THE NATURE OF THE POLYHEDRAL BODIES FOUND IN INSECTS.[1]

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Contents.

Introduction.

A very large group of lepidopterous larvæ is subject to a class of infectious diseases known as the "polyhedral diseases." Whether the organism concerned in their production is identical or not for all the species of insects affected must remain a matter of conjecture till further work allows us to venture an interpretation. One thing, however, is certain, namely that curious crystal-like structures called "polyhedral bodies" or "polyhedra" are always associated with the type of diseases we are here discussing. Although these polyhedra may vary considerably in size and somewhat in shape in the different species of insects, nevertheless, they are always specific for a certain type of malady.

Wahl, followed by Prowazek and Escherich, consider the polyhedral diseases as distinct and absolutely divorce them from the fungous, protozoan and bacterial affections of insects. We believe that the erection of a separate group to embrace all of the polyhedral diseases is an excellent plan and receives our sympathetic endorsement, for the reason that the confusion of all of the insect diseases is still common amongst entomologists. We venture to say that there are scarcely two entomologists in America who know the difference or similarity between any of the diseases expressed by such terms as Muscardine, Pébrine,

[1] Contribution from the entomological laboratory of the Bussey Institution in coöperation with the U. S. Bureau of Entomology (Bussey Institution No. 115).

flacherie, lethargia, maladie de morts-blancs, schlaffsucht, faulsucht, fettsucht, polyhedral disease, wilt, wipfelkrankheit, jaundice and gelbsucht.

We do not wish to dwell upon the differences or similarities between all of these maladies, but will confine ourselves solely to the group of polyhedral diseases under which are included the four commonest manifestations, viz., wilt, wipfelkrankheit, jaundice and gelbsucht. Wilt is the vernacular term used in America for the polyhedral diseases. It is a name suggestive of post-mortem aspects, but unfortunately a number of caterpillar diseases distinct from wilt on superficial examination also have a similar post-mortem appearance. Moreover, a number of diseases common to plant pathology are labelled with the same term. We do not think it wise to eliminate the name, however, for the reason that it has been used so long and "wilt" certainly means more to field entomologists and foresters than "polyhedral disease" which is only significant to laboratory workers. The term "Wipfelkrankheit," which is used for a similar affection of nun moth caterpillars in Germany, is a very suggestive name for the reason that when the animals are in the last stages of the disease, they congregate in masses at the tops of the trees or "wipfeln" and die hanging by their prolegs. The name "Wipfelkrankheit" has the further advantage in that it is not applied to any other plant or animal disease. "Gelbsucht" or "jaundice" are terms used to designate a polyhedral disease in silkworms. The two terms are descriptive of the clinical picture of the diseased worms and are not used for any other affection known to pathology.

The larval stages of the following species of lepidoptera have been examined by us and found to be susceptible to the polyhedral diseases. Animals which can only be infected experimentally have been omitted, *i. e.*, the list comprises only those which besides being capable of experimental infection also have the disease or diseases in a state of nature.

I. Saturnidæ.

1. *Hemileuca maia* Drur.

II. Arctiidæ.

2. *Apantesis virgo* Linn.

III. Noctuidæ.
  3. *Leucania unipuncta* Halw.
  4. *Noctua clandestina* Harris.
  5. *Autographa brassicæ* Riley.
IV. Lymantridæ.
  6. *Porthetria dispar* L.
  7. *Lymantria monacha* L.
  8. *Orgyia leucostigma* S. and A.
V. Lasiocampidæ.
  9. *Malacosoma americanum* Fabr.
  10. *Malacosoma disstria* Hubn.
VI. Bombycidæ.
  11. *Bombyx mori* L.
VII. Dioptidæ.
  12. *Phryganidia californica* Packard.
VIII. Pieridæ.
  13. *Colias philodice* Godart.

From a perusal of the above list it will be seen that the polyhedral diseases are very widely distributed and affect some of our most important economic insects. We have estimated that epidemics of polyhedral diseases at certain times kill off from 30 to 70 per cent. of some of our most noxious pests. This is especially true, as we have found in connection with our studies on the gipsy moth, tent caterpillars and army worms. The polyhedral diseases contribute much more to the control of certain of our noxious caterpillars than the combined efforts of all their hymenopterous and dipterous parasites. Therefore, we believe that these diseases merit a serious consideration from all points of view.

The Polyhedral Bodies.

Caterpillars dead from wilt are usually found on some elevated place hanging by their prolegs. Dead nun moth caterpillars are found hanging from the very highest branches of a conifer, dead gipsy moth caterpillars are found hanging anywhere on the trunk of a tree or on a branch; the favorite dying places of the American tent caterpillars being usually in close proximity to the nest on the branches of an apple or cherry tree. The army

worm seeks the tip of a grass blade and succumbs thereon. An innate desire to reach an elevated place on their favorite food plant always seizes the diseased insects prior to death. We have never observed animals in the last stages of wilt descend their food plants. So far we are unable to offer any explanation which would satisfactorily assist in analyzing this ascending instinct. A short time after death, the animals become deliquescent. At the slightest touch the skin ruptures and a dark brown liquid oozes out. In some species such as the American and forest tent caterpillars this liquid is pink shortly after death and becomes dark brown later. The corpses will be practically odorless if they have hung but a short time and before septic bacteria have gained a foothold.

If some of the brown liquid from a dead caterpillar is examined microscopically with a high-power dry or oil-immersion lens, it will be found to contain, besides the elements of disorganized tissues, myriads of polyhedral bodies of various sizes. (Fig. 1, Plate 1.) Certain polyhedra have been found to measure $\frac{1}{2}\,\mu$ and less in diameter while still others reach the size of $15\,\mu$. The average polyhedron of the gipsy moth caterpillar measures $3.4\,\mu$ in diameter. The bodies in this species are larger than those in any other form we have hitherto examined. The average size of nun moth caterpillar polyhedra measure $2.65\,\mu$ in diameter; those of the forest tent caterpillar $2.6\,\mu$ and so on until we come to *Phyrganidia californica* and the tussock moth caterpillars in which the average diameter has been found to be $1.6\,\mu$ and $1.5\,\mu$ respectively. Thus it is seen that the average polyhedron varies greatly in size in the different species. As stated previously the sizes of the polyhedra within one species or even within one animal (gipsy moth: $\frac{1}{2}\,\mu$–$15\,\mu$) varies also. There exists a striking similarity between the shapes of these bodies in the different species but some variation within a particular species or even within the same animal can be observed. In general the form is that of a polyhedron with more or less rounded angles. They never assume the shape of a perfect sphere, and an actual geometric outline has never been observed except in the silk worms where almost perfect octahedra are found. The polyhedra are highly refractive, and on focusing are seen to have a

denser center differentiated from a somewhat lighter periphery. Sometimes within the bodies concentric layers like those of an onion are observable. Often two polyhedra are seen adhering to one another as if in the act of dividing, but an actual division in a hanging-drop has never been observed. When pressure is applied to the cover glass, the polyhedra crack very readily into a number of pieces, and often without the application of pressure the same fragmentation may be observed to occur somewhat more slowly. In the latter case a notch appears at one side of the polyhedron which gradually lengthens into a line progressing slowly toward the other side, much like the cracking of ice. Usually before the line has completely separated the two halves other lines appear, and soon the entire polyhedron is divided into a number of pieces, which may separate or may stick together in a rosette-like fashion. At no time was anything observed to come out of the polyhedra when they cracked in this manner. If the cover glass is moved while applying a little pressure, one half of the polyhedron may sometimes be folded upon the other half without the cracks appearing, showing that it is composed of a tough substance and is not at all brittle like inorganic crystals.

The only objects in a fresh preparation with which one could possibly confuse the polyhedra are the fat globules and urate crystals, but with a little practice these may be readily distinguished. Fat globules are perfectly spherical and are therefore unlike the polyhedral shape of the bodies in question; but when in doubt, Sudan III was used, for in this stain the fat globules become red, while the polyhedra remain colorless. The urate crystals are often more acutely angular or are of an entirely different shape from the polyhedra and are frequently traversed by radiating lines.

Besides polyhedral bodies, fat globules, and urates, a smear from a newly "wilted" caterpillar contains cellular débris, hairs and pigment granules. The pigment granules must not be confused with bacteria, for many of them superficially resemble these organisms very closely. When a preparation is dried, mounted, and examined under oil, the pigment granules of the gipsy moth may easily be confused with small micrococci, owing to the fact that they are usually arranged in pairs. As a

matter of fact, a smear made from a recently wilted caterpillar is almost devoid of bacteria, and in many cases none at all can be found. If bacteria are present, they have escaped into the body cavity through rupture of the intestine and bear no direct etiological relation to the disease.

In fixed and stained smears a number of things can be demonstrated to advantage within the polyhedra. Fixation was accomplished either by passing the preparation through a flame or by placing it in absolute alcohol for a few minutes. The smears were then stained in Giemsa's solution for 12 hours or were stained for a shorter period with one of the following dyes: Methylene blue, trypan blue, gentian violet, carbol fuchsin, Bismarck brown, or iron hæmatoxylin. When iron hæmatoxylin was used, the preparation was first mordanted in a 4 per cent. ferric-alum solution for two or three hours. After staining, the preparations were sometimes quickly passed through the alcohols to xylol before mounting. This not only clears everything, but dissolves away all the fat on the slide and thus increases the transparency of the preparation. Gipsy moth polyhedra are rather resistant to stains in general and usually color along the periphery only, unless the stain is applied for a long time. When this is done one may succeed in staining the entire polyhedron, especially after the use of some mordant like ferric alum before hematoxylin or anilin water before gentian violet. Steaming the preparation with a stain like carbol fuchsin has also given good results. When properly stained, one of three conditions is observed: First, the polyhedral bodies are uniformly stained so that nothing can be detected within them; or second, a uniformly darker staining central mass can easily be differentiated from an almost unstained outer substance; or, third, many little refractive, reddish granules are seen within the polyhedra. An actual differentiation between what might be interpreted as nuclear and cytoplasmic material within the polyhedra never occurs. Therefore, in accounting for the staining reactions we believe that at times the polyhedra have a central granular or homogeneous substance easily distinguishable from an outer tougher substance which is more resistant to the dyes. This varies a great deal, however, and sometimes the periphery takes the

stain more readily than the underlying strata. From these staining reactions it becomes apparent that the polyhedra are complicated in structure, and do not therefore differ essentially from what Bolle and Prowazek found to be true of the silkworm polyhedra. Our observations on the staining reactions also show that morphological studies do not enable us to regard the polyhedral bodies as organisms. We believe that the polyhedra are protein degeneration-products of the disease. The staining reactions have demonstrated that they are not simple crystals, but complicated in structure, and have a tough outer layer. Consequently, and for a number of other reasons, we do not believe them to be true crystals and therefore choose to call them pseudo-crystals. The variations in their staining reactions which one obtains at times can well be accounted for by assuming that one is dealing with different stages in the synthetic process of pseudo-crystals. Another matter militating against the idea that the polyhedra are organisms is the fact that Glaser ('15) and Chapman and Glaser ('16) have experimentally demonstrated the possibility of infecting healthy gipsy moth caterpillars with wilt material from which the polyhedral bodies were removed by passing the virus through Berkefeld Grade "N" candles. We have shown that wilt is caused by a filterable virus and believe that the polyhedra arise as a reaction against the invasion of this virus.

Origin of the Polyhedral Bodies.

Studies on sectioned gipsy moth, army worm and tent caterpillar material have shown that the polyhedra originate within the nuclei of the hypodermal, fat, tracheal matrix, and blood cells. (Fig. 2.) In the true army worm, however, polyhedra are at times formed within the nuclei of intestinal epithelial cells. We have been utterly unable to find the bodies within the nuclei of muscle tissue, Malpighian tubes, ganglia, nerves, œnocytes, salivary glands, gonads and within the intestinal epithelial cells of all forms except the true army worm.

The formation of the polyhedral bodies within the nuclei of the four tissues above mentioned and the visible changes taking place within these nuclei may be described as follows: The first

indication of a diseased nucleus seems to consist in the flowing together of the chromatin into a lump in the middle. Then out of the achromatic substance the polyhedra arise as very minute individuals. (Fig. 2, Plate I.) They gradually increase in size and probably most of the chromatin is also used up during the synthetic process. As the polyhedra grow, they become more and more refractive, stain with difficulty, and the nucleus becomes hypertrophied. The late stages of these hypertrophied nuclei are more than twice as large as the largest normal nucleus. (Fig. 2, Plate 1, and 3, 4 and 5, Plate II.) This swelling of the nucleus is due to the increase in size of the polyhedral bodies which stretch the nuclear membrane. During the earlier stages of the disease the polyhedra are somewhat rounder than the larger ones found later on prior to death. This can be accounted for by the fact that, as the polyhedra grow, they become so closely packed within a nucleus that they press upon one another and thus the more or less polygonal shape is produced. As the polyhedra grow and become more refractive the remains of the chromatin lump disappears and there remains simply the nuclear membrane enclosing the polyhedra. (Fig. 2, Plate 1, and 3, Plate II.) Finally the nucleus disintegrates, and the polyhedra are found free in great numbers in smears of dead caterpillars. (Fig. 6, Plate II.) We believe (and this belief is based on morphological, chemical and experimental evidence) that the polyhedral bodies are degeneration-products of the disease—products of nuclear disintegration. This view may not seem so improbable if one reviews some of the literature dealing with a few of the diseases in higher animals.

Hydrophobia (Rabies).

In 1903, Negri described certain bodies occurring in the nervous system of animals dying of rabies. The bodies seem to be specific to the disease, and are of great assistance for diagnostic purposes. The Negri bodies vary in size, measuring from .5 to 25 μ. They are round, oval or angular in outline and are found in the protoplasm of the nerve cells and their processes. The bodies occur in all parts of the nervous system, but are most common in the Purkinje cells of the cerebellum and especially in the cells of the cornu Ammonis. The virus of hydrophobia passes through

the coarser Berkefeld and Chamberland filters, and these filters exclude the Negri bodies. The most generally accepted view at present is that the Negri body is a cellular reaction against the invasion of the filterable virus. The virus of hydrophobia has not been cultivated.

Variola and Vaccinia (Smallpox and Cowpox).

In 1892 Guarnieri described certain cellular inclusions in variola and vaccinia which are specific for these two diseases. The bodies are from 1–8 μ in diameter, and round, oval, or sickle-shaped. They lie in the cellular spaces, often in close proximity to the nucleus, and can be demonstrated in vaccine pustules as well as in the experimental lesions produced in the rabbit's cornea. The virus of variola and vaccinia passes through the coarser porcelain (Chamberland) filters. Most authorities regard the Guarnieri bodies as the effect of a specific reaction of epithelial cells against the virus. The virus has not been cultivated.

Trachoma.

In trachoma certain granules are found in the cytoplasm of the inflamed epithelial cells which cover the conjunctiva. Bodies similar to the trachoma bodies have also been found in other inflammations of the conjunctiva, and are therefore thought not to be specific of trachoma. The trachoma bodies are regarded as the product of mucous secretion under pathological conditions. The virus passes through Berkefeld filters. It has not been cultivated.

Cellular inclusions not regarded as parasitic by some workers have been found in a number of other diseases. In scarlet fever cellular inclusions occur in the skin lesons; in foot and mouth disease inclusions are found in the vesicles; in fowl pest inclusions are found in the brain and in epithelioma contagiosum or fowl diphtheria in the epithelium. Cytoplasmic inclusions accompanying many of the diseases of higher animals, and regarded as non-parasitic in most cases are not at all uncommon, but we have been unable to find any account in the literature of vertebrate diseases which are accompanied by the formation of *nuclear* inclusions as is the case with the polyhedral diseases of insects.

Bio-chemical Observations on the Polyhedral Bodies.

According to Tubeuf, Krassilschtschik, Prowazek, Wahl, Wolff, Glaser and Chapman the polyhedral bodies are regarded as being reaction products; towards bacteria (Tubeuf, Krassilschtschik) or towards Chlamydozoa (Prowazek, Wolff) or towards an unknown virus (Wahl) or towards a filterable virus (Glaser and Chapman). According to Bolle, Fischer, Marzocchi, and Knoche the polyhedral bodies are stages of a protozoan; according to Escherich and Miyajima they are bearers of an unknown virus.

It seems that the views are very diverse as regards the true nature of the polyhedra. For this reason it was thought advisable to submit some of our bio-chemical work on this subject in further support of our contention, obtained from morphological and experimental studies, that the polyhedra are merely organic degeneration-products of the disease.

During the gipsy moth season great quantities of diseased material can be obtained. For this reason all of the bio-chemical investigations were performed with gipsy moth polyhedra. These bodies are heavier than water and consequently can be obtained in bulk by centrifuging aqueous emulsions of diseased material. By repeated washing, filtering and centrifuging most of the fat, cellular débris, etc., can be eliminated. After this treatment the polyhedra were always washed with ether in order to free them from any possible remnants of adhering fat. Thorough attention to this cleansing operation will yield polyhedra in a fairly pure state for chemical tests. The material was allowed to dry naturally in the centrifuge tube, after which the lump that formed at the bottom could be loosened and transferred to a mortar where it was pulverized. This pulverization if done gently does not crack or injure the polyhedra in any way. After this procedure the mass of polyhedra look very much like pulverized chalk. It is comparatively easy to obtain 2 or 3 grams of polyhedra from about one or two hundred caterpillar cadavers.

As the polyhedra do not blacken with osmic acid, and do not stain with Sudan III., it seems unlikely that they contain fat. They stain with picric acid, however, and this gave us the clue to their possible protein nature. The color tests for dry proteins

were then applied and we obtained positive reactions with xanthoproteic, Millon's, biuret, Adamkiewicz's and Lieberman's tests. It was next found necessary to obtain the polyhedra in solution so that the various coagulation or precipitation tests could be performed.

Before testing the solubility of the polyhedra in various reagents they were first rubbed energetically in an agate mortar with the addition of a little sea sand. This grinding was found necessary for the reason that the outer surface of the bodies is composed of more resistant material than the underlying strata. By grinding with sea sand the polyhedra are fragmented and this offers more delicate surfaces to the action of the reagents.

The polyhedra were found to be insoluble in hot or cold water, alcohol, chloroform, ether, or xylol. They dissolve readily in strong acids and alkalies, but these reagents were thought to produce too great a hydrolytic cleavage of the protein molecule, and since we did not wish to alter our material to any appreciable extent a number of milder reagents were tried. Moreover, from the standpoint of the classification of proteins it is important to determine just what will and what will not dissolve the material.

The following solubility tests were performed. Two grams of ground polyhedra were divided into four parts. To ½ gram water was added; to ½ gram .5 per cent. and to another ½ gram 2 per cent. NaCl solution, and to the fourth ½ gram 10 per cent. Na_2CO_3 were added. The tests were kept over a water bath for 13½ hours at a temperature varying between 55° and 58° C. At the end of this time the solutions were filtered and the various tests for soluble proteins applied. All of the tests (acetic acid, nitric acid, cupric sulphate, mercuric chloride, acetic acid with potassium ferrocyanide and ammonium sulphate) were negative showing that nothing went into solution.

Two grams of polyhedral material were again divided into four parts and treated respectively with H_2O, .5 per cent. and 2 per cent. NaCl and 10 per cent. Na_2CO_3. The tests were placed over a small direct flame for two hours. At the end of this time the solutions were filtered and the tests for soluble proteins applied. Negative tests were obtained with the water and salt

solutions. The material treated with the 10 per cent. Na_2CO_3 solution gave slight coagulation tests with acetic and nitric acids showing that a small amount of protein went into solution. The polyhedra treated with the carbonate were examined microscopically and it was found that some fragments had partially dissolved while most of the polyhedra, which had apparently resisted fragmentation in the mortar had swollen to double their normal size.

Concentrated HCl (37 per cent.) used both hot and cold seems to dissolve the polyhedra with difficulty. The solubility of the bodies in boiling HNO_3 seems to lie between 15 and 20 per cent. We began with 4 per cent. HNO_3 in which the polyhedra are not affected and worked up to 31 per cent. HNO_3 in which they dissolve instantaneously on boiling. The fact that the liquid clears when some of the lower percentages of HNO_3 are used is not sufficient evidence that all of the polyhedra have been dissolved. For this reason the solubility of the bodies towards the acid was checked by microscopical examinations.

$(NH)_4OH$ does not seem to affect the polyhedra, but the other alkalies such as KOH and NaOH dissolve them readily. It was found that as low a percentage as 1/16 per cent. NaOH will dissolve polyhedra if they are boiled in the solution. For convenience 2 per cent. NaOH was used for the following tests. Two grams of ground bodies were dissolved in the alkali by means of heat. The solution was then dialyzed in order to get rid of the alkali. The dialysis was usually complete after 24 to 48 hours. At the end of this procedure the proteins remain in solution (*i. e.*, on dissolving in alkali, after which, although the alkali be removed, the polyhedra proteins remain soluble) and the tests for soluble proteins can be applied. We obtained positive reactions with acetic acid, nitric acid, cupric sulphate, mercuric chloride, acetic acid with potassium ferrocyanide and ammonium sulphate.

It might be well to mention that the percentages of NaOH used were accurate and the material pure. In making up the solution we did not rely on the so-called purity of the hydroxide sticks, but always eliminated every trace of Na_2CO_3 by precipitation with $Ba(OH)_2$.

After determining that the polyhedra are protein in nature it

was next thought advisable to ascertain whether or not they are nucleoproteins. It seemed likely that this would be the case for the reason that they are formed in the nuclei of certain tissue cells. Two grams of whole (unrubbed) polyhedra were digested for one week in artificial gastric juice. After this time a microscopic examination failed to reveal any polyhedra. Theoretically, everything should have been decomposed excepting the nucleins which have a high phosphorus content. At the end of one week's digestion the material was filtered through and dried on ash free filter paper. The paper containing the residue was then cut into fine pieces and put into a platinum crucible with an oxidizing mixture (Na_2CO_3 (2 parts) + KNO_3 (1 part)). The material was slowly ignited and the residue dissolved in weak HNO_3. This solution was then warmed with the addition of some NH_4NO_3 in order to make the expected precipitate less soluble. Lastly 5 per cent. molybdic acid was added. The material was placed in an incubator and on standing a pronounced yellow precipitate (ammonium-phospho molybdate) was formed.

On the basis of this and the other tests described the polyhedra meet all of the requirements of the nucleoproteins. Nucleoproteins give all the color reactions, are soluble in water containing a small amount of alkali (1/16 per cent. in case of polyhedra) and are precipitated from this solution by acetic acid. The nucleins which have a high phosphorus content are not decomposed by gastric juice, and are obtained as an insoluble residue after the artificial digestion of nucleoproteins with pepsin.

Since iron is an element known to be contained in chromatin it was further thought advisable to determine whether the polyhedra during their synthesis from the chromatin and other substances in the nuclei embodied any iron. It will be needless to go through all the details of the analysis. Suffice it to say that every precaution to prevent iron contamination from water, air, etc., was used. Reagents known to be free from iron contamination were employed. Furthermore, the polyhedra were not rubbed with sand for fear of introducing iron in this manner. .0988 of a gram of polyhedra were used and .00049 of a gram of iron was found.

On dissolving polyhedra in alkali and after dialyzing away

the alkali, three fractional precipitations with magnesium sulphate or sodium chloride can be obtained. Whether this means that the polyhedra are composed of three separate proteins or three groups of proteins, we are not prepared to say. The three precipitates, however, do demonstrate that the polyhedra are complex and not at all simple, a fact which does not seem strange when one reflects on the complexity of cellular or nuclear material in general.

As stated previously we regard the polyhedra as degeneration products formed during the course of the disease in the nuclei of certain tissue cells. The bodies are nucleoprotein crystal-like (pseudo-crystalline) aggregates. The idea suggested itself to us that it might be possible to dissolve the polyhedra and recrystallize them again after dissolution. If this should prove to be possible, it would militate seriously against the views held by Bolle, Fischer, Marzocchi, Knoche, Escherich and Miyajima that the bodies are organisms or the stages of an organism. Our experimental attempts at recrystallizing the polyhedra are a bit varied and so we do not wish to overemphasize our results as yet, but submit them with a full appreciation of their preliminary value.

Polyhedra were dissolved in 2 per cent. NaOH by heating. The process of dissolution was followed by an examination of samples microscopically, and when traces of the bodies could no longer be observed, the material was evaporated over a water bath and examined before it became entirely dry. Besides long Na_2CO_3 crystals we found many small and large bodies which resembled polyhedra. As a check we evaporated ordinary 2 per cent. NaOH, but we could not find the polyhedra-like crystals obtained with the protein solution. This seemed to be a result which offered possibilities, so we proceeded more carefully with another experiment.

Two grams of polyhedra were dissolved by heating in 2 per cent. NaOH. This solution was filtered and washed with ether in order to rid the material of any traces of fat. The ether was then eliminated by means of a separating funnel. The solution was next dialyzed to get rid of the alkali and a few protein tests were performed with some of the solution just to convince our-

selves of the presence of proteins. This protein solution freed from the alkali was now slowly evaporated. On partial evaporation, we found beautiful single and double crystals which simulated the polyhedra very closely. If the material is evaporated completely it is difficult to find the crystals owing to the presence of coagulated and other protein material which hides them. If one shoots water under the cover-slip, however, the crystals again become visible just as soon as the coagulated sediment softens and becomes transparent. This fact that a coagulated residue remains shows that the entire protein material contained in the original polyhedra is not used during the formation of these new crystals. The majority of the crystals produced in this way simulate polyhedra very closely, but some are rounder and larger. Double forms are very common and are absolutely indistinguishable microscopically from ordinary polyhedra. The staining reactions of the crystals are similar to those of the polyhedra and Millon's reaction is identical. We have as yet not obtained a sufficient amount of these new crystals to submit them to all of the protein tests applied to the polyhedra. The crystals are not quite as stable as the polyhedra. They seem to lack the more resistant outer layer and therefore are more easily soluble in alkali and other reagents. For this and other reasons we do not claim to have reproduced typical polyhedra after their disintegration, but we firmly believe that the results are suggestive. It seems unreasonable, after submitting proteins to the violent hydrolytic action of both heat and alkali, to expect to reproduce the identical proteins. However, in the material under consideration, there seems to be a tendency for this particular protein or group of proteins to crystallize out in the shape characteristic of the polyhedra. These observations seem to support our view that the polyhedra are merely degeneration-products and not some inexplicable, unclassifiable organisms as supposed by many workers. An organism certainly could not be dissolved and its original form again reproduced or very nearly reproduced on evaporation.

So far we are unable to obtain the crystals after the dissolution of the polyhedra at every trial. Out of possibly ten trials, one usually succeeds four or five times. Undoubtedly some condition

of which we are at present ignorant is responsible for the frequent failures. We have performed a sufficient number (15) of these recrystallization experiments, however, to warrant a report of the results.

Crystallizable proteins are, of course, not uncommon. Hæmoglobin is perhaps the best known example in animals and the aleurin grains a well known example in plants. By fractional precipitation with magnesium sulphate or sodium chloride two or three separate crystalline proteins can be obtained from the albumen of the hen's egg. In insects, by the evaporation of blood with a trace of acetic acid, beautiful protein crystals can be obtained, different in every species.

Our view regarding the nature of the polyhedral bodies may therefore be summarized as follows: During the course of the disease the virus disintegrates the nuclear material in such a way that crystal-like bodies called polyhedral bodies or polyhedra are synthesized out of the disintegrating proteins. Just how the process from nuclear material to polyhedra takes place is at present unknown. At any rate, from our morphological observations, experimental infection data (published elsewhere) and from our chemical studies here presented, it seems clear that the polyhedra are nucleoprotein degeneration-products and not organisms responsible for a series of insect diseases.

Summary.

1. Polyhedral bodies are found in many different species of lepidopterous larvæ.

2. The bodies are specific for a certain type of disease.

3. The polyhedra vary in size in the different species.

4. There exists a striking similarity in shape between the polyhedra found in different species.

5. The polyhedra are structurally complicated.

6. They arise in the nuclei of certain tissue cells.

7. Cytoplasmic inclusions are found in certain diseases of higher animals.

8. Nuclear inclusions have not been known previously.

9. The polyhedra are nucleoprotein crystal-like degeneration-products and not organisms.

10. The polyhedra contain iron and phosphorus.

11. On dissolving polyhedra in alkali and after dialyzing away the alkali and evaporating the protein solution crystals are obtained which simulate the original polyhedra.

BIBLIOGRAPHY.

Chapman, J. W. and Glaser, R. W.

'15 A Preliminary List of Insects which Have Wilt, with a Comparative Study of their Polyhedra. Journal Econ. Ent., Vol. 8, No. 1, pp. 140–149, 1 pl.

'16 Further Studies on Wilt of Gipsy Moth Caterpillars. Journal Econ. Ent., Vol. 9, No. 1, pp. 149–167. Tables 21.

Escherich, K. and Miyajima, M.

'11 Studien über die Wipfelkrankheit der Nonne. In Naturw. Ztschr. Forst u-. Landw. Jahrg. 9, Heft 9, s. 381–402, 6 fig.

Glaser, R. W. and Chapman, J. W.

'13 The Wilt Disease of Gipsy Moth Caterpillars. Journal Econ. Ent., Vol. 6, No. 6, pp. 478–488.

Glaser, R. W.

'14 The Bacterial Diseases of Insects. Psyche, Vol. 21, No. 6, pp. 184–190.

'15 Wilt of Gipsy Moth Caterpillars. Journal of Agri. Research, Vol. 4, No. 2, pp. 101–128, 17 fig., 4 pl.

Huntemüller

'13 Filtrierbare Virusarten. Ztschr. f. Chemotherapie, Bd. 2, Heft 1, pp. 56–69, Tafeln 9.

Osborne, Thomas B.

'99 Crystallized Vegetable Proteids. Am. Chem. Jour., Vol. 14, No. 8, pp. 1–28, fig. 3.

'99 Egg Albumin. Jour. of Am. Chem. Society, Vol. 21, No. 6, pp. 477–485

'99 On Some Definite Compounds of Protein-bodies. Jour. of Am. Chem. Society, Vol. 21, No. 6, pp. 486–493.

Osborne, Thomas B., and Campbell, George F.

'00 The Proteids of the Egg Yolk. Twenty-third Report of the Conn. Agri. Ex. Sta., pp. 339–348.

'00 The Protein Constituents of the Egg White. Twenty-third Report of Conn. Agri. Ex. Sta., pp. 348–375.

Osborne, Thomas B., and Harris, Isaac F.

'03 The Precipitation Limits with Ammonium Sulphate of Some Vegetable Proteins. Jour. of Am. Chem. Society, Vol. 25, No. 8, pp. 837–842.

Prowazek, S. von.

'07 Chlamydozoa. II. Gelbsucht der Seidenraupen. In Arch. Protistenk., Bd. 10, Heft 2/3, s. 359–364, 2 fig.

'12 Untersuchungen über die Gelbsucht der Seidenraupen. In Centbl. Bakt. (etc.), Abt. 1, Orig., Bd. 67, Heft 4, s. 268–284, 1 fig., 2 pl.

'12 Gelbsucht (Polyederkrankheit) der Raupen. In Prowazek's Handbuch der Pathogenen Protozoen, Bd. I., s. 156–161, 2 fig., Verlag von Johann Barth. Leipzig, 1912.

Tubeuf, Carl von.

'92 Die Krankheiten der Nonne. (Liparis monacha.) In Forstl. Naturw. Ztschr., Jahrg. 1, Heft 1, s. 34–37, fig. 9–16; Heft 2, s. 62–79.

'92 Weitere Beobachtungen über die Krankheiten der Nonne. In Forstl. Naturw. Ztschr., Jahrg. 1, Heft 7, s. 277–279.

'11 Zur Geschichte der Nonnenkrankheit. In Naturw. Ztschr. [Forst u-. Landw., Jahrg., 9, Heft 8, s. 357–377.

Wolbach, S. B.

'12 The Filterable Viruses, a Summary. Jour. of Med. Research, Vol. 27, No. 1 (New Series, Vol. 22, No. 1), pp. 1–25.

'11 The Nature of Trachoma Bodies. Jour. Med. Research, N. S., Vol. 19, No. 2, pp. 259–264. 3 pl.

Wolff, M.

'10 Ueber eine neue Krankheit der Raupe von Bupalus piniarius L. In mitteilung des Kaiser Wilhelm Institutes für Landwirtschaft in Bromberg, Bd. III, Heft 2, s. 69–92.

Wahl, Bruno.

'09–'12 Ueber die Polyederkrankheit der Nonne (Lymantria monacha L.). In Centbl. Gesam. Forstw., Jahrg. 35, Heft 4, s. 164–172; Heft 5, s. 212–215; Jahrg. 36, Heft 5, s. 193–212; Heft 8/9, s. 377–397; Jahrg. 37, Heft 6, s. 247–268; Jahrg. 38, Heft 8/9, s. 355–378.

PLATE I.

FIG. 1. Photomicrograph of a smear showing free polyhedra.

FIG. 2. Photomicrograph showing various stages during the formation of polyhedra in tissue nuclei of a gipsy moth caterpillar. × 720.

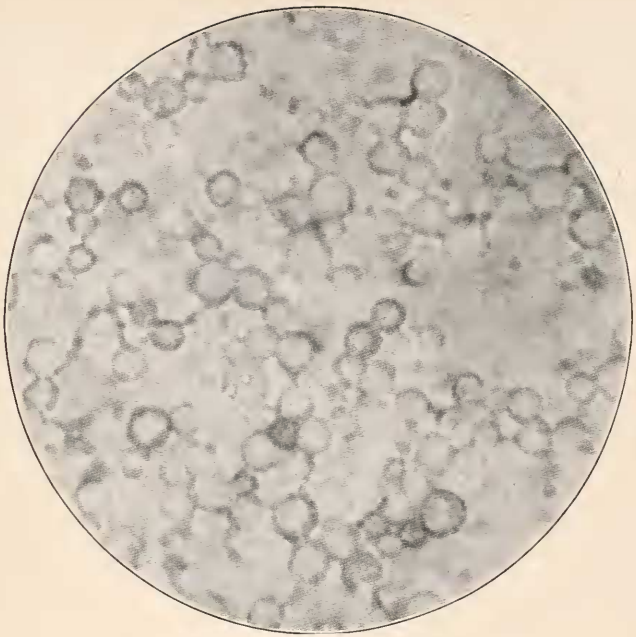


FIG. 1.

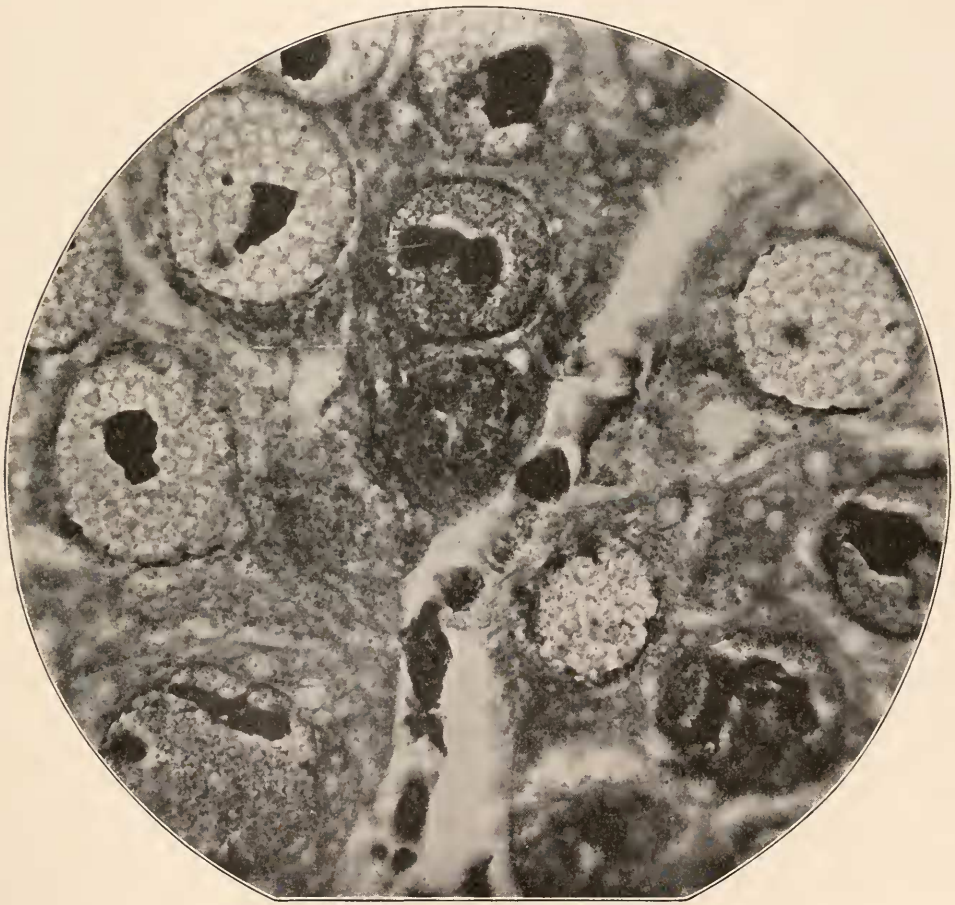


FIG. 2.

PLATE II.

FIG. 3. Photomicrograph of army worm tissue showing fully developed polyhedra in nuclei of fat cells. × 300.

FIG. 4. Photomicrograph of army worm tissue showing normal hypodermal, fat, muscle and blood cells. × 300.

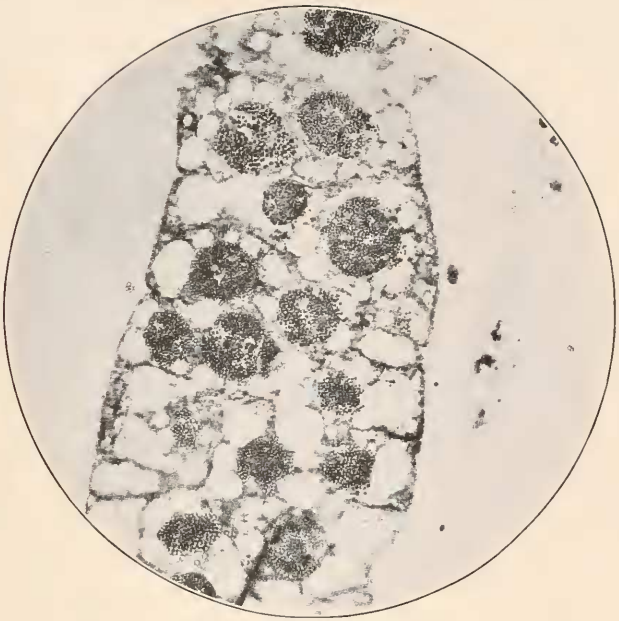


FIG. 3.

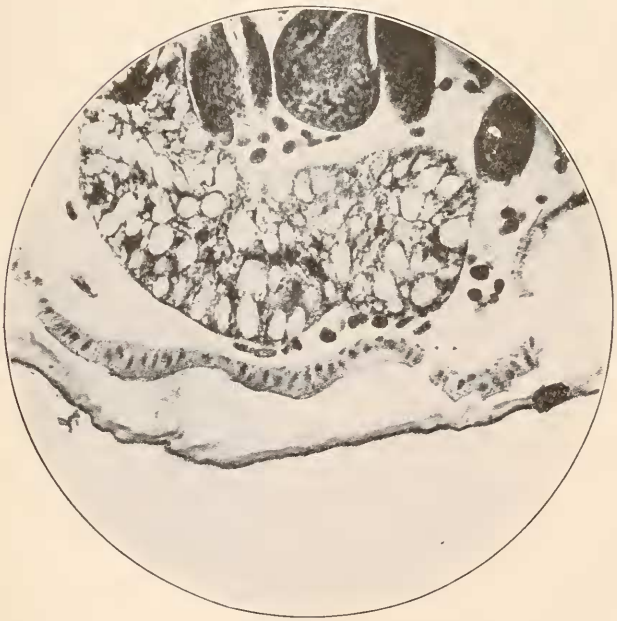


FIG. 4.

PLATE III.

FIG. 5. Photomicrograph of army worm tissue showing normal, hypodermal, fat, muscle and blood cells. × 300.

FIG. 6. Photomicrograph of army worm tissue showing disintegration of nuclei and cells with liberation of polyhedra. × 300.

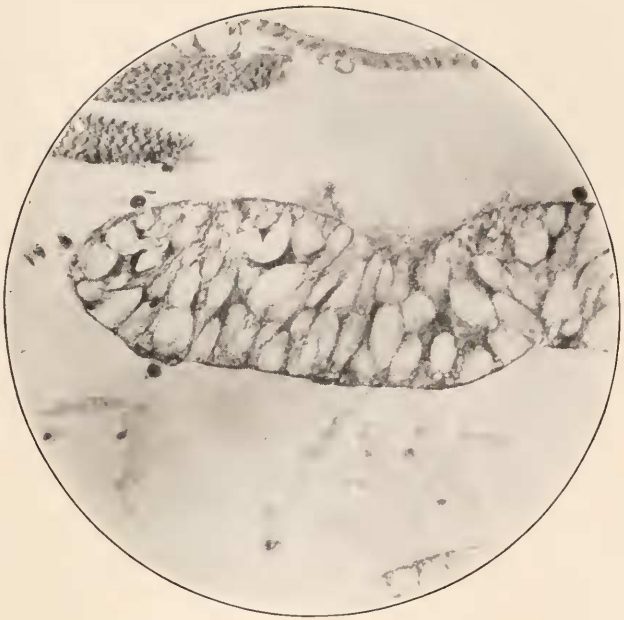


FIG. 5.

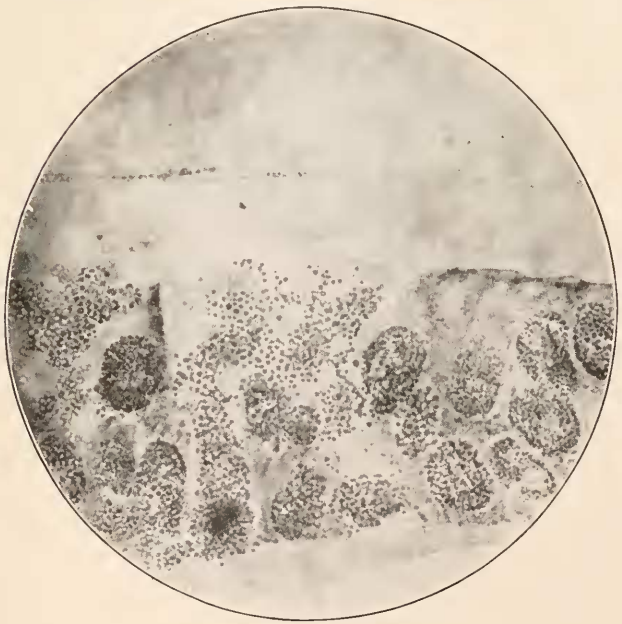


FIG. 6.

AXIAL SUSCEPTIBILITY GRADIENTS IN THE EARLY DEVELOPMENT OF THE SEA URCHIN.

C. M. CHILD.

(With 20 Figures.)

Axial gradients in susceptibility to cyanides and various other agents have already been demonstrated in *Planaria* (Child, 13*b*) various infusoria (Child, '14), the early developmental stages of the starfish (Child, '15*a*), in a number of species of Oligochetes (Hyman, '16), in a number of algæ and in various other animals the data for which are as yet unpublished. Thus far such gradients have been found, at least in the earlier stages of development in all forms examined, comprising more than sixty species and including algæ, cœlenterates, flatworms, echinoderms, annelids, and vertebrates. The relation of these gradients to developmental gradients of other kinds and the problem of their significance for the physiological individual has also been considered (Child, '15*b*, Pt. III, '15*c*).

During the summers of 1913 and 1915 at the Marine Biological Laboratory, Woods Hole, Mass., axial susceptibility gradients were demonstrated in the early developmental stages of the sea urchin, *Arbacia punctulata*, and the control and modification of development by means of the differential susceptibility along these gradients was found to be possible. In the present paper only the direct evidence for the existence of such gradients, based on the progress of death and disintegration along the axis, is considered and this is incomplete in certain respects. The indirect evidence from the control and modification of development, with which I was chiefly concerned and which is of greater interest, will be presented in another paper.

Methods of Demonstrating Susceptibility Gradients.

The method employed in demonstrating the gradients has already been described (Child, '13*a*, '15*b*, Chap. III.) and consists in directly determining the differences in susceptibility along

the axis or axes to cyanides and various other agents used in concentrations sufficient to kill in the course of a few hours, but not high enough to kill immediately and not low enough to permit the organisms to become acclimated or acquire a tolerance to them. The agents used in these studies on the sea urchin were potassium cyanide, ammonium hydrate, ethyl alcohol and hydrochloric acid in sea water. The various developmental stages were placed in concentrations of these substances determined by preliminary experiment and the progress of death along the axis was observed.

In many of the lower animals the progress of swelling, cytolysis, separation and disintegration serves directly as an indication of the progress of death. In the developmental stages of the sea urchin changes of this sort occur as the cells die, but they differ somewhat with different reagents and different stages of development. In KCN the cells swell, become spherical, and separate from each other as they die and the region concerned breaks down into a shapeless mass of these spherical cells which soon disintegrate. If motor activity is still present in other parts the dead cells may be progressively left behind as the living portion moves about. This disintegration of the body is more marked in the earlier stages of the blastula and gastrula than in later stages where supporting tissues have differentiated, but even in the later stages extensive disintegration can be brought about by return to sea water after a sufficient length of time in KCN. These death changes are somewhat accelerated and intensified by the return to water and death is marked by very complete disintegration. Thus when KCN is used, the progress of death can either be followed directly under the microscope in the KCN solution, at least in the blastula and gastrula stages, or lots may be returned to water at stated intervals and the progress of death determined by the comparison of dead and living portions of the body in successive lots. Both methods have been used, but the latter is more satisfactory in many cases, because after return to water the dead portions disintegrate rapidly and completely while the parts which are still alive may recover and resume motor activity where the stages in which movement occurs are concerned. The progress of death can also be made

visible by staining with neutral red before placing in KCN. When death occurs the neutral red color changes to yellow, as the alkali of the KCN solution penetrates the cells, and then disappears.

The death changes in ammonium hydrate are very similar to those in KCN. The cells swell and in the blastula and gastrula stages separate, but in later stages the cells cohere more or less after death and the visible death changes are merely swelling and rounding of the cells and a consequent increase in size and greater translucency of the whole. Here likewise, return to water increases the disintegration and so makes it easier to follow the progress of death, and neutral red may also be used as an indicator of death in the same way as with KCN.

Ethyl alcohol is used in the same way as KCN and NH_4OH, but of course in much higher concentration. Disintegration of blastula and gastrula stages is very complete and in the plutei only the supporting tissues retain the body form.

In hydrochloric acid, however, the behavior of the cells is different, as might be expected. The cells shrink and do not separate and death may occur with very little visible change except in size. But return to sea water after a sufficient length of time in HCl brings about disintegration and the dead and dying cells swell and separate so that the progress of death can be followed without difficulty by removing lots from HCl to sea water at regular intervals.

As regards concentration of the reagents used, a wide range is possible according to the stage of development and the length of survival time desired. Since the susceptibility increases very greatly from fertilization up to the blastula stage as physiological rejuvenescence occurs (Child, '15*b*, pp. 412–418), much higher concentrations can be used for the former than for the latter stages. At a temperature of 22–24° C. unfertilized eggs begin to die after 4–5 hours in KCN *m*/100, while blastulæ and later stages begin to die after the same length of time in KCN *m*/1,000. The other reagents were used only on the blastula and later stages. In NH_4OH *m*/500 death of these stages begins in half an hour to an hour, in *m*/1,000 in 1–2 hours. The same stages in alcohol 4 per cent. (roughly *m* 2/3) begin to die in 3–4

hours and after 5–10 minutes in HCl *m*/400 death begins on return to water, but it is difficult to determine just when it occurs, if the stages are left in HCl. Much lower concentrations of KCN and NH_4OH can be used without the occurrence of any appreciable degree of acclimation, but acclimation to alcohol and HCl takes place much more rapidly and in relatively high concentrations, alcohol 1.5 per cent., HCl *m*/2,000, so that for direct demonstration of the susceptibility gradient with these reagents the range of concentration is not so great.

The significance of differences in susceptibility to cyanides, various narcotics and certain other agents has been considered elsewhere (Child, '13*a*, '15*b*, Chap. III.). It has been found that in concentrations high enough to kill without acclimation the susceptibility varies in general directly with the rate of metabolism or of certain fundamental reactions, while in concentrations low enough to permit acclimation the higher the metabolic rate the greater the degree and rapidity of acclimation consequently in the long run the susceptibility to these concentrations varies inversely as the metabolic rate.

Although the susceptibility method has proved in certain cases to be a very satisfactory means of distinguishing differences in general metabolic activity, it is actually of course a rather crude method and merely makes it possible to compare in a rough way metabolic differences in different individuals or body regions and does not of course give us any exact quantitative data. Moreover, the nature of the method makes it evident that we cannot expect to distinguish with certainty the minuter metabolic differences, because the reagents used decrease to some extent the differences which they are expected to show. Definite and constant differences in susceptibility must mean considerable differences in rate of reaction, but the absence of such differences in susceptibility does not necessarily mean the complete absence of differences in rate of reaction. Notwithstanding these limitations, however, the method is useful and the positive results obtained by means of it afford ample proof of its value.

One further point requires some consideration. The method has been criticized, more particularly in personal conversation, as involving certain assumptions concerning the action on living

chemically active protoplasm of cyanides and other agents used. This, however, is not the case. There is no reason for believing that different agents which retard or inhibit metabolic activity in living protoplasm all act in the same way. Protoplasm is a complex system in which both the physical substratum and the chemical reactions play a part, but the important point is that it is a system, *i. e.*, that the different processes, changes and conditions in it are not independent of each other but mutually correlated and dependent to a greater or less degree. It is not in the least improbable that different agents may give the same general results, as regards susceptibility, even though one acts primarily on the aggregate condition of the colloids or let us say the permeability of membranes, another on the production or constitution of enzymes, and still another on the chemical reactions of oxidation. My observations on susceptibility to cyanides, various narcotics, acids, alkalies, metabolic products, and even temperature have convinced me that the relation between susceptibility to retarding or inhibiting agents and conditions and general metabolic rate or the rate of certain fundamental metabolic reactions is a very general relation and there seems to be absolutely no reason for believing that it is dependent upon any one particular method of action on the protoplasmic system of the agent or condition employed. It still remains of course for future investigation to determine the exact method of action of each agent and condition, to formulate the general rule and to discover and account for exceptions if they exist. Since our present knowledge indicates both that the oxidations are fundamental metabolic reactions and that they are more or less dependent on various conditions in the protoplasmic system; we may expect to find that their rate is altered by a great variety of external agents and conditions even though these do not enter directly into the chemical reactions of oxidation.

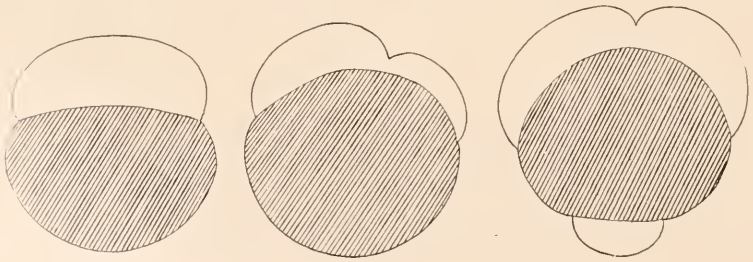
In certain cases, as in the green plants, the energy for certain of the synthetic or anabolic reactions is derived directly from sources outside the organism and in such cases these reactions may be to a considerable degree independent of the energy-producing reactions in the organism. Such reactions, however,

are in a sense only preliminary, although of course essential to the fundamental processes of life and the relation between susceptibility and metabolic rate is primarily concerned, not with them, but rather with the reactions which play a fundamental rôle in setting free the energy characteristic of living organisms.

In the sea urchin the differences in susceptibility along the axis so far as observed, are essentially the same whether cyanides, alcohol, alkalies or acids are used as reagents. All these substances interfere with the action of the protoplasmic system in some way, but by no means necessarily in the same way, yet the general results as regards susceptibility are the same. This fact is highly significant and indicates to some extent the general character of the relation between susceptibility and the fundamental conditions and processes of life.

The Eggs and Cleavage Stages.

Since experiments on the control and modification of development through differential susceptibility along the axes had shown very conclusively the existence of axial gradients in the early developmental stages and had indicated their probable presence even in the unfertilized egg, and since it was soon found that the character of the death changes made it difficult to reach definite conclusions concerning death gradients from direct observation, but little time was spent on these earlier stages and the evidence obtained, so far as it has any value, is merely contributory. I regret to state, however, that I neglected to employ as a means of orientation the method of making the micropyle visible by colored suspensions in the water. This might have made the data on these earlier stages somewhat more conclusive.



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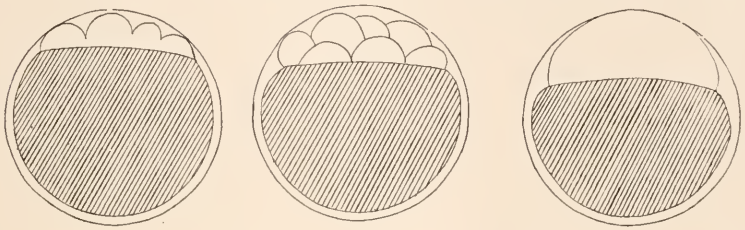
The unfertilized eggs in KCN $m/100$ show death changes of the character indicated in Figs. 1–3. First, one or more clear droplets or masses which contain some colloids and much water appear on the surface of the egg and grow larger, while the granular portion decreases in size. This process may go on, the clear masses often uniting, until the clear and granular portions are almost equal in size, but finally the granules spread and the



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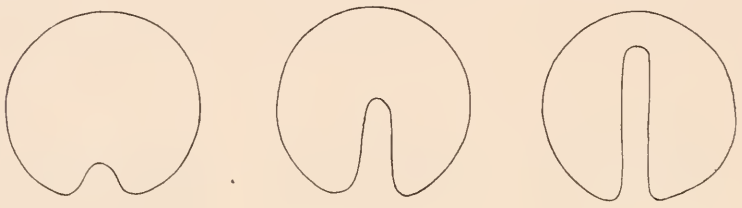
distinction between clear and granular areas disappears. The only indication of a susceptibility gradient is the appearance of the clear droplets only or earlier on one hemisphere or at one pole. Within certain limits of concentration this has been observed in some 70–80 per cent. of the eggs, but in some eggs the clear droplets appear at opposite poles or over various parts of the surface at the same time. The fertilized eggs before cleavage (Figs. 4–6) behave in KCN essentially like the unfertilized.

The blastomeres of the earlier cleavages show a similar separation into clear and granular areas and here again the clear areas or droplets usually appear first or only on one hemisphere or at one pole of the egg. In eggs placed in KCN after the appearance of the micromeres I believe that the apical hemisphere is in general more susceptible than the basal.

An interesting gradient in the first cleavage appears in certain concentrations of cyanide. In such cases the first cleavage plane at its first appearance extends as a furrow only half way or less around the egg and cuts through the egg in one direction without the appearance of any furrow on the opposite side (Figs. 7–10). In some cases a larger or smaller portion of the cytoplasm is separated from the two blastomeres at the end of this cleavage

by the division of the cleavage furrow into two (Fig. 11). Such cleavages occurred in about 40 per cent. of eggs placed in KCN $m/1{,}000$ for $10\frac{1}{2}$ hours before fertilization then well washed and kept in sea water and fertilized one hour after removal from KCN. Figs. 7–10 are drawn from such eggs. After this treatment there



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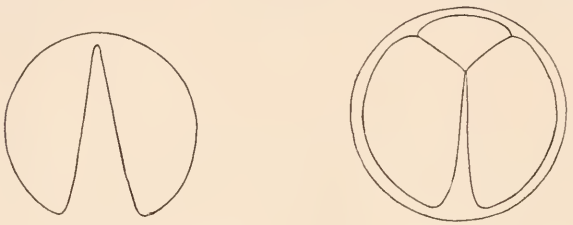

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is no elevation of a fertilization membrane from the egg surface, and the blastomeres frequently become entirely separated after cleavage. Eggs placed in KCN $m/100$ fifteen minutes after fertilization and washed and returned to sea water after three hours also showed cleavage of this kind in 30–40 per cent. In the other eggs of these lots cleavage is normal or incomplete and often irregular or else delayed with simultaneous formation of a number of blastomeres. Apparently then these one-sided cleavages represent the first step in departure from normal cleavage. The indirect evidence shows that a susceptibility gradient is present in these stages of development and that the region of highest susceptibility and therefore of highest metabolic rate is the apical pole. It is evident that cyanide inhibits cleavage, and if the apical pole is most susceptible, we should expect to find cleavage most completely inhibited there, and least inhibited at the basal pole. It is probable, therefore, that these cleavage furrows start from the basal and proceed toward the apical pole,

and that in cases like that in Fig. 11, where a portion of the cytoplasm is cut off, it is the apical region, which is so much injured that it cannot give rise to a cleavage furrow.

With the same methods of treatment a general gradation in the size of the blastomeres was observed in 30–40 per cent. of the eggs in stages from 32 cells onward, though it became less marked in later stages as recovery proceeded. In these cases also it is probable that the region of most rapid cleavage and therefore of the smallest blastomeres is the basal region and the region where cleavage is most inhibited and therefore the blastomeres are largest is the apical region.

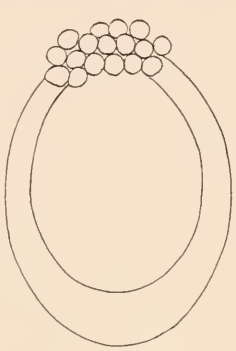
The small size and protoplasmic character of the micromeres at the basal pole suggests the possibility that their metabolic rate may be higher than that of the cells about them, but I have not been able to discover that their susceptibility is greater than that of the adjoining cells, and the indirect evidence shows that the mesenchyme cells, which are believed to be at least in part descendants of the micromeres are among the least susceptible if not the least susceptible of all the cells.

These observations on the earlier developmental stages are not conclusive. Taken by themselves they are of little value, but considered in the light of the indirect evidence and of the more definite results obtained in later stages they possess a certain significance as contributory evidence. My observations on these stages were only incidental to other work and only KCN was used as reagent.

The Blastula and Gastrula.

As soon as the blastula begins to elongate the direction of movement, and in more advanced stages the greater thickness of the cellular wall in the basal region make it possible to distinguish apical and basal ends without difficulty. In these stages death begins at the apical end and proceeds basally (Figs. 12–15), advancing in many cases somewhat more rapidly down one side (Figs. 13, 14), probably that side which later becomes the anterior end of the pluteus. The progress of death in the basal direction is very regular, though a few cells here and there may swell and push out of the body-wall earlier than others

about them. The susceptibility gradient is the same with all the reagents and methods of use noted in the preceding section so that there can be no doubt of the existence of an apico-basal gradient which is fundamentally related to the activity of the



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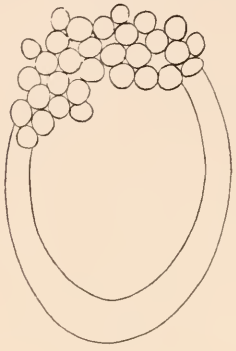

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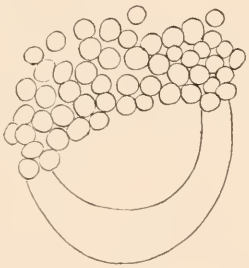
living protoplasm. The more rapid progress of death down one side of the blastula which is frequently observed is probably an indication of differences in other axes, but on this point certainty is impossible.



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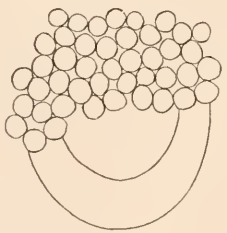

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In the gastrula stage the same gradient appears (Figs. 16–18), the apex of the gastrula, which represents the apical region of the egg and blastula, being most susceptible, the basal least susceptible. The susceptibility of the entoderm and the blastopore region is very much lower than that of the other ectodermal regions. The entoderm is still intact after practically the whole ectoderm has disintegrated (Fig. 18) and in concentrations where

ectodermal disintegration occurs in three or four hours disintegration of the entoderm is usually not complete until one or two hours later or in some cases even a longer time.

By returning to sea water after the proper length of time in the

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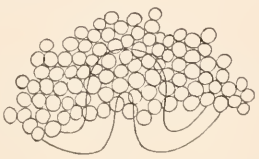

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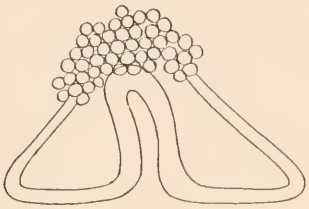
reagent it is possible to stop death at any level of the body and to bring about recovery and further development of the parts still alive. In the blastula it is more or less of the basal portion which remains alive, and this may close up and gastrulate, producing dwarf gastrulæ with a disproportionately large enteron. In the gastrula stage also partial dwarf gastrulæ with large enteron result from partial disintegration and recovery, because the more apical ectoderm may be in large part destroyed while the basal region of the gastrula and the entoderm remain intact. The forms of larvæ which develop from these partial basal blastulæ and gastrulæ will be described in another paper.

Later Stages.

In the early stages of transformation into the pluteus, the gastrula loses its apparent radial symmetry and becomes triangular in outline in basal or anal view, the base of the triangle representing the anterior region of the future pluteus. In side view the apex of the gastrula is seen to be shifted toward the anterior end as compared with earlier stages (*cf.* Figs. 19 and 16). In the further transformation this apical region becomes the oral lobe of the pluteus and the long anal arms develop from the basal region of the anterior end (Fig. 20).

The susceptibility gradient in the earlier stages of this transformation is the same as in the gastrula, apico-basal (Fig. 19) and the same difference in susceptibility between ectoderm and entoderm persists. Later, when the larva begins to elongate in



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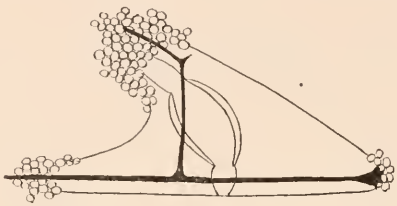

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the antero-posterior direction, and the anal arms begin to develop, these arms and the posterior end both appear as secondary regions of high susceptibility (Fig. 20) though the susceptibility of the anal arms is in general somewhat less than that of the oral lobe and that of the posterior end somewhat less than that of the anal arms. On the oral lobe and over the body death progresses in the basal and posterior direction and in the anal arms from tip to base of the arms.

In the fully developed pluteus the susceptibility gradients are less marked. The ectoderm of the oral lobe and anal arms is still somewhat more susceptible than that of other regions but the differences are less conspicuous. In all these later stages, however, the entoderm remains much less susceptible than the ectoderm and apparently the mesenchyme is least susceptible of all parts.

It is probable that the gradual fading out of the metabolic gradients in the pluteus is a physiological change which precedes and makes possible the development of the axial gradients of the mature sea urchin which have been previously inhibited by the existing axial relations. If this suggestion is correct these changes are the factors which determine, or rather permit metamorphosis.

The development of the arms and the posterior elongation has been shown to be dependent on the development of the skeleton. That being the case the appearance of high susceptibility in the anal arms and the posterior end is not self-determined in these parts but results from skeletal growth.

Discussion and Summary.

The existence in the apico-basal axis of the blastula, gastrula and later stages of a definite and conspicuous gradient in susceptibility which is the same for cyanide, alcohol, acid and alkali is a significant fact, particularly in the light of the relations between susceptibility to cyanides and various narcotics and general metabolic rate. The results with HCl and NH_4OH show that this relation is the same for these substances. The further evidence for the existence of this gradient in the earlier developmental stages, together with the difficulty of conceiving how an apico-basal gradient could arise *de novo* during the earlier development, leave little doubt that this axial gradient persists from the unfertilized egg to the mature pluteus. This conclusion will be confirmed by the indirect evidence presented elsewhere.

As regards gradients in other axes the evidence is less conclusive. It is probable that the asymmetry in the progress of death frequently observed in the blastula (Figs. 13, 14) is an indication of a difference between anterior and posterior regions and this probability is strengthened by the appearance of a similar asymmetry in the gastrula and prepluteus stages (Figs. 17 and 19).

It must be remembered, however, that this method of determining susceptibility is far from being a perfect method for the demonstration of differences in metabolic rate. It can be expected to show only the grosser differences, for the reagents used tend to decrease the differences which they are used to demonstrate, and if the differences are originally slight they may disappear so rapidly in the reagent that no appreciable or constant differences in the time of death appear.

It is evident from the course of development that the apico-basal gradient is the most strongly marked and in the early stages even this is not very clearly defined by differences in susceptibility as determined by time of death, though it becomes more distinct later. It is not to be expected that the minor differences along the axes of symmetry should appear as clearly by this method as the differences along the major axis. The important facts are that the major axis appears so distinctly as

a susceptibility gradient and that indications of susceptibility differences in the minor axes have been observed.

The apico-basal susceptibility gradient is the same as that in the starfish (Child, '15*a*) and in all other forms examined, at least in early developmental stages and in many cases throughout life. That this gradient is of fundamental significance cannot be doubted and I have attempted elsewhere (Child, 15*c*) to present some of the evidence which seems to me to indicate that a physiological axis is fundamentally such a gradient in metabolic rate.

The chief points are summarized as follows:

1. A distinct gradient in susceptibility to potassium cyanide, ethyl alcohol, ammonium hydrate and hydrochloric acid is present along the apico-basal axis of blastula, gastrula, and later stages of larval development of *Arbacia* and indications of a gradient are found in earlier stages.

2. In the apico-basal gradient the susceptibility is highest at the apical end of the axis and lowest at the basal end. Since susceptibility to these reagents varies in general directly with metabolic rate, the susceptibility gradient indicates the existence of a gradient in rate of metabolic activity in which the rate is highest in the apical region and decreases basally.

3. Some indications of gradients in other axes appear in the differences of susceptibility, but these are much less distinct than the differences along the apico-basal axis.

4. The anal arms and the posterior end of the larval body appear as secondary regions of high susceptibility after they begin to develop.

5. In the fully developed pluteus these susceptibility gradients become less marked and doubtless disappear as metamorphosis begins.

Hull Zoological Laboratory,
University of Chicago,
February, 1916.

REFERENCES.

Child, C. M.

'13a Studies on the Dynamics of Morphogenesis and Inheritance in Experimental Reproduction. V. The Relation Between Resistance to Depressing Agents and Rate of Reaction in Planaria dorotocephala and its Value as a Method of Investigation. Jour. Exp. Zool., XIV.

'13b Studies, etc. VI. The Nature of the Axial Gradients in Planaria and their !Relation to Antero-posterior Dominance, Polarity and Symmetry. Arch. f. Entwickelungsmech., XXXVII.

'14 The Axial Gradient in Ciliate Infusoria. BIOL. BULL., XXVI.

'15a Axial Gradients in the Early Development of the Starfish. Amer. Jour. Physiol., XXXVII.

'15b Senescence and Rejuvenescence. Chicago.

'15c Individuality in Organisms. Chicago.

Hyman, L. H.

'16 An Analysis of the Process of Regeneration in Certain Microdrilous Oligochetes. Jour. Exp. Zool., XX, 2.

Vol. XXX. *June, 1916.* *No. 6*

BIOLOGICAL BULLETIN

THE MARINE BIOLOGICAL LABORATORY

EIGHTEENTH REPORT; FOR THE YEAR 1915

TWENTY-EIGHTH YEAR.

I. TRUSTEES

EX OFFICIO

FRANK R. LILLIE, *Director*, The University of Chicago.
GILMAN A. DREW, *Assistant Director and Vice-President*, Marine Biological Laboratory.
D. BLAKELY HOAR, *Treasurer*, 161 Devonshire Street, Boston, Mass.
GARY N. CALKINS, *Clerk of the Corporation*, Columbia University.

TO SERVE UNTIL 1918

CORNELIA M. CLAPP ..... Mount Holyoke College.
E. G. CONKLIN ..... Princeton University.
ROSS G. HARRISON ..... Yale University.
CAMILLUS G. KIDDER ..... 27 William Street, New York City.

M. M. METCALF.....Oberlin, Ohio.
WILLIAM PATTEN.....Dartmouth College.
JACOB REIGHARD.....University of Michigan.
W. B. SCOTT.....Princeton University.

TO SERVE UNTIL 1917

S. F. CLARKE.....Williams College.
CHARLES A. COOLIDGE....Ames Building, Boston, Mass.
C. R. CRANE.....Woods Hole, Mass., *President of the Board.*
ALFRED G. MAYER.....Carnegie Institution.
C. E. MCCLUNG.....University of Pennsylvania.
T. H. MORGAN.....Columbia University.
ERWIN F. SMITH.....United States Department of Agriculture.
E. B. WILSON.....Columbia University.

TO SERVE UNTIL 1916

H. H. DONALDSON.....Wistar Institute of Anatomy and Biology.
M. J. GREENMAN.....Wistar Institute of Anatomy and Biology.
C. W. HARGITT.....Syracuse University.
H. S. JENNINGS.....Johns Hopkins University.
GEORGE LEFEVRE.....University of Missouri, *Secretary of the Board.*
A. P. MATHEWS.....The University of Chicago.
G. H. PARKER.....Harvard University.
HENRY B. WARD.....University of Illinois.

TO SERVE UNTIL 1915

H. C. BUMPUS.....Tufts College.
R. A. HARPER.....Columbia University.
W. A. LOCY.....Northwestern University.
JACQUES LOEB.....The Rockefeller Institute for Medical Research.
F. P. MALL.....Johns Hopkins University.
GEORGE T. MOORE.....Missouri Botanical Garden, St. Louis.
L. L. NUNN.....Telluride, Colo.
JOHN C. PHILLIPS.....299 Berkeley Street, Boston, Mass.

II. ACT OF INCORPORATION

No. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns,

Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our LORD ONE THOUSAND, EIGHT HUNDRED and EIGHTY-EIGHT.

HENRY B. PIERCE,
Secretary of the Commonwealth.

[SEAL.]

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio*, members of the Board of Trustees, and Tustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio*.

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. TREASURER'S REPORT

Inasmuch as the records of the Laboratory have been reorganized on an income and expense basis, taking into account the bills receivable and payable, as well as the cash receipts and disbursements, the treasurer makes a departure from the form of report previously employed in order to be in accord with the new methods.

The cash statement of operations is, therefore, now presented in consolidated form and the income and expense schedule follows accompanied by a balance-sheet, showing the condition of the Laboratory on December 31, 1915, as far as the figures are available. Figures for the plant and inventory valuations are in process of compilation.

Consolidated Cash Statement of Receipts and Disbursements for the Year ended December 31, 1915

Operating Accounts

Cash on hand January 1, 1915			$1,686.68
Receipts from departments		$46,676.99	
Receipts from donations		35,500.00	
Receipts from work done for others		1,402.16	
Receipts from loans repaid		125.00	
Total receipts for year		$83,704.15	
Payments for departmental expenses	$61,856.38		
Payments for improvements	16,863.51		
Payments for work done for others	1,537.05		
Payments for new town landing	761.50		
Payments for Loan	25.00		
Total payments		81,043.44	
Excess of receipts for year			2,660.71
			$4,347.39
Less Oklahoma warrant included in cash receipts but not yet paid			135.23
Cash balance December 31, 1915			$4,212.16

Cash Receipts and Payments on Account of Investments For the Year ended December 31, 1915

Reserve Fund

Cash on hand January 1, 1915	$210.30	
Receipts:		
Interest —$3,000 American Telephone & Telegraph Company, 4 per cent bonds	60.00	
¾ of $500 Western Telephone & Telegraph Company 5's	18.74	
Dividend—6 shares American Smelting & Refining Company, preferred	42.00	
8 shares General Electric Company	64.00	
14 shares United Shoe Machinery Corporation, preferred	21.00	
2 shares Massachusetts Gas, preferred	8.00	
Interest on bank deposit	1.04	
	$425.08	
Payments:		
Purchase of 2 shares Massachusetts Gas Companies, preferred	180.25	$244.83

Lucretia Crocker Fund

Cash on hand January 1, 1915	$ 99.04	
Receipts:		
Interest —1/5 of $1,000 American Telephone & Telegraph Company 4 per cent. bond	8.00	
Dividend—18 shares Vermont & Massachusetts Railroad Company	108.00	
1 share West End Street Railway Company	3.50	
1 share American Telephone & Telegraph Company	8.00	
2½ shares General Electric Company	20.00	
	$246.54	
Payments:		
2 Scholarships	100.00	146.54

Library Fund

Cash on hand January 1, 1915	$174.16	
Receipts:		
Interest —4/5 of $1,000 American Telephone & Telegraph Company 4's	32.00	
¼ of $500 Western Telephone & & Telegraph Company, 5's	6.26	
Dividends—3 shares American Telephone & Telegraph Company	24.00	
1 share American Smelting & Refining Company, preferred	7.00	
2½ shares General Electric Company	20.00	
5 shares United Shoe Machinery Company, preferred	7.51	
2 shares Massachusetts Gas Companies, preferred	8.00	
	$278.93	
Payments:		
Purchase 2 shares Massachusetts Gas Companies, preferred	181.26	97.67
Cash on hand December 31, 1915		$489.04

Income and Expense for Year ended December 31, 1915

	Expense	*Income*
Administration expense	$ 6,805.36	
Biological Bulletin	2,594.69	$ 1,523.36
Boat department expense	6,629.63	
Carpenter department expense	983.09	
Chemical department expense	1,430.82	
Dormitories	1,489.49	1,691.04
Fish trap	1,159.21	667.21
Instruction	3,435.98	5,150.00
Philosophical lectures	100.00	
Library department expense	2,628.64	
Mess	15,753.01	16,165.48
Membership dues		1,000.00

Maintenance, buildings and grounds	5,220.23	
New laboratory expense	1,927.76	
Pumping station expense	464.70	
Research income		3,175.00
Sundry expense and income	2,165.48	3,255.25
Supply department	12,822.40	19,150.77
Interest on notes payable	150.00	
Total expense	$65,760.49	
Total income	51,778.11	$51,778.11
Excess of expense	$13,982.38	
Contribution by Mr. C. R. Crane	20,000.00	
Excess carried to balancing account	$ 6,017.62	

BALANCE-SHEET, DECEMBER 31, 1915

Assets		*Liabilities and Capital*	
Cash, bank	$ 4,212.16	Accounts payable	$ 1,394.02
Petty cash	200.00	Note payable	3,000.00
Accounts receivable	7,642.37	Trust funds	8,222.55
Investments	10,733.51	Balancing account	33,997.98
Investment cash	489.04		
Inventories			
Plant account			
Improvements, 1915	23,337.47		
	$46,614.55		$46,614.55

D. BLAKELY HOAR, ESQ., Treasurer,
Marine Biological Laboratory
Woods Hole, Mass.
161 Devonshire Street, Boston.

Dear Sir: We have audited the accounts of the Marine Biological Laboratory as kept at Woods Hole and of the trust funds and accounts as kept at your office, 161 Devonshire Street, for the year ended December 31, 1915.

We have checked the report of the Treasurer submitted above and find it correct and in accord with the books. The extent of the audit together with the supporting schedule is set forth in a detailed report under date of February 1, 1916.

Very respectfully,

HARVEY S. CHASE & CO.,
Certified Public Accountants

V. LIBRARIAN'S REPORT

August, 1915

Since the last annual report the reorganization of the library has been carried on by Miss Scott, who has brought each department up to a state of system and efficiency.

We can now make a more definite statement of the contents of the library, including a number of accessions not previously listed: Total number of accessions 10,046. Old and new accessions recorded since last report 6,746. Of these there have been added since 1914:

934 volumes loaned by the American Museum of Natural History,
466 volumes given by the Wisconsin Academy of Sciences,
200 " given by Mr. Crane,
720 " bound during year.

These volumes may be conveniently divided as follows:

Journals, for which most of our appropriation is expended and which form the bulk of the library, in bound sets	95
Serials and publications of academies, museums, laboratories, and societies (many of these are from exchanges which are steadily increasing)	188
Books:	
Zoology	321
Botany	134
Physiology	100
Hygiene and medicine	74
Chemistry	18
Evolution	82
Psychology	36
Philosophy	104
Miscellaneous	198
Total	1,067

We can buy very few books and are dependent upon gifts from authors and publishers, who recognize that this is a particularly useful place to have their works examined.

The separates number 4,000. Our reprint collection might easily be notably increased through the aid of authors and friends. Such duplicates are in great demand.

The following advances have been made. First, and very important, is the considerable number of missing parts which

have been secured by Miss Scott's enterprise toward completing back volumes of sets.

These have been completed: *Nature, Wilson Bulletin, University of California Publications in Zoölogy, Colorado University Studies, Bergens Museum Publications.*

These have been added to but are still incomplete:

Annales d. Sciences Naturelles—Zoölogy,
Bulletin Museum of Comparative Zoölogy, Harvard,
Proceedings Boston Society of Natural History,
Proceedings American Philosophical Society,
American Naturalist,
American Journal of Science and Arts,
Journal Medical Research,
Field Columbian Museum Publications,
Bureau of Fisheries, Bulletin and Report,
Contributions U. S. National Herbarium,
Proceedings American Association for the Advancement of Science,
Proceedings Iowa Academy of Sciences,
Proceedings Indiana Academy of Sciences.

The following new sets have been added to the library:

Transactions American Microscopical Society,
Transactions Wisconsin Academy of Sciences,
Bulletin Torrey Botanical Club,
American Journal of Botany,
Proceedings Academy of Natural Sciences, Philadelphia,
Journal of Parasitology,
Illinois Biological Monographs,
Unpopular Review,
Annals Missouri Botanical Garden,
Indiana University Studies,
U. S. Bureau of Education, Report and Bulletin,
U. S. War Department, Bulletin,
Maine Agricultural Experiment Station, Report and Bulletin,
Museum of Brooklyn Institute of Arts and Sciences, Science Bulletin.

Seventy-six volumes of Hoppe-Seyler's *Zeitschrift für Physiologische Chemie* were purchased from Miss Koch for $200, and

the remainder of the set, which is now in the 94th volume, has been secured.

Exchanges now number 29; six have been arranged this year: *Indian Museum; American Journal of Botany; New York Academy of Sciences; Academy of Natural Sciences, Philadelphia; Biologische Untersuchungen, Stockholm;* and *Zoologiska Bidrag*, Upsala. New subscriptions have been placed for the *Popular Science Monthly* and the *English Journal of Physiology.*

Here we must report a partial disposition of the Journal Fund which was begun in 1912. Since then, a number have subscribed to this fund which is to enable us to secure new files of journals. Drs. Mayer, Knower, Meigs, Rice and Just have subscribed from $5 to $10 a year for a period of five years. This has already furnished $55 and will still accumulate from new gifts.

In addition, last year Mr. Tashiro and a number of other investigators, interested in securing the *English Journal of Physiology*, subscribed amounts from 50 cents to $1 each, thus securing $7.50. This sum has now been increased to $12. For this, we must thank Drs. Tashiro, Heilbrunn, Gould, Wherry, Packard, Sturtevant, Bridges, Harvey, Metz, and Morgan, and Misses Hoge, Medes, Browne, and Dunn. Such coöperation is most encouraging. It makes possible a purchase long needed. We have subscribed to the *Journal* with this gift and have used the amount already accumulated for the Journal Fund to purchase a number of back volumes, hoping gradually complete to the set.

Further growth in this spirit of coöperation in building up the library is shown in the list of important gifts.

Various publishers have given a total of 16 books this year. Miss Fay has given several quite valuable books on mushrooms. Mr. Crane gave us two hundred volumes from his personal library, as well as two very interesting Russian curios. Dr. George T. Moore gave duplicates from his library, 28 bound volumes and 21 separates. Dr. Philipp Fischelis donated 53 valuable papers by Kowalewsky and other Russian authors. Dr. Conklin has given his book on "Heredity and Environment," Dr. Parker, his book on "Biology and Social Problems," and Dr. Abbott has ordered a copy of his book on "General Biology" sent to us. Dr. Calkins also presented his texbook on "Biology."

The Wistar Institute gave a set of the "Biological Lectures," Mrs. Gardiner donating Volume I. to complete our set. Dr. Ward gave a subscription to the *Journal of Parasitology*, and Dr. Bumpus to the current volume of the *American Naturalist* and the *Unpopular Review*. Dr. Gustav Retzius has promised to send a complete set of his works as soon as transportation is safer. Three hundred and twelve reprints have been presented to the library this year by various authors.

Through the coöperation of Dr. George Wagner, a large number of duplicates from the library of the Wisconsin Academy of Sciences are being transferred to this library; 466 volumes have been received and the number will probably reach 1,000.

The Prince of Monaco has sent us about 75 photographs of the buildings and equipment of the Institut Oceanographique, together with pamphlets telling about the foundation and progress of the institute. A number of other stations have sent various literature and photographs, and it is hoped to secure material of this kind from all biological stations in the world.

We wish to acknowledge the coöperation of Drs. Moore and Duggar in securing the *Bulletin of the Torrey Botanical Club*, and of Dr. Ivey Lewis in arranging and preparing for the binder a most confusing set of plates.

We are really in need of a number of journals that we do not have, and we should complete as soon as possible certain sets that have long been defective. The library needs an increased appropriation for handbooks, monographs, and general works like the Encyclopedia Britannica, the Century Dictionary, etc.

Above all, we must point out the great value of reprints and books sent here by individuals, and the importance of personal efforts in behalf of the library on the part of the biologists who use it. Such interest has already done much to build it up.

VI. THE DIRECTOR'S REPORT

To the Trustees of the Marine Biological Laboratory:

Gentlemen: The season of 1915, the twenty-eighth session of the Marine Biological Laboratory, was marked by a farther

advance in many of the statistics of the Laboratory as will appear from the appended lists (1) of the Staff, (2) of the Investigators and Students, (3) the Tabular View of Attendance, (4) the Subscribing Institutions, (5) of the Evening Lectures, (6) of the Members of the Corporation. The Treasurer's report and the Librarian's report also show a healthy condition of growth. We have much cause for congratulation, and reason for deep thankfulness to Mr. Crane without whose whole-hearted financial support and intelligent sympathy with our objects, our best efforts must have been comparatively ineffective.

The number of investigators in attendance was 137 as compared with 129 in 1914, 122 in 1913 and 93 in 1912; the number of students was 105 as compared with 89 in 1914, 69 in 1913 and 67 in 1912. The total attendance was 242 as compared with 218 in 1914, 191 in 1913 and 160 in 1912. These figures bear testimony to the growing appreciation of the facilities furnished by the Laboratory both in research and in instruction. The number of institutions represented by these workers was 79, which is almost the same as in the two preceding years; the increase was therefore due to larger attendance from certain institutions. Indeed no considerable increase in the number of institutions represented is to be expected, because practically all of the larger institutions of higher education in the East and Middle West are represented each year, and the variations from year to year are accounted for by fluctuations of a more or less fortuitous character among the smaller institutions represented.

The receipts from subscribing institutions and fees were $8,325 as compared with $7,300 in 1914, $6,160 in 1913 and $5,175 in 1912. The Supply Department has also shown an encouraging increase, having filled 282 more orders than in 1914 with total paid receipts of $16,932.00 as compared with $14,003.35 in 1914.

Three important additions to the property and equipment of the Laboratory were made during the year: (1) The so-called Bake House property adjoining the Laboratory property on the east was purchased; this piece has about 103 feet frontage on the Eel pond and on the main street by 100 feet in depth, and its title carries with it the private roadway previously controlled jointly by the Laboratory and the owners of this property. The

old house on the property has been fitted up as a workshop for the carpenters' and plumbers' department, which was very badly needed. The frontage on the eel pond is especially valuable for future development. (2) The Laboratory also purchased the grounds and house known as the Ritter property, 78½ by 100 feet, adjacent to the Whitman house, and opposite the lecture hall. The house furnished much needed enlargement of our dormitory facilities. (3) The old "Homestead" hitherto used as the matron's and helpers' house, adjoining the mess, was torn down and replaced by a much larger, modern and attractive dwelling house with accommodations for 43 persons. As all of this space was not needed for the mess, part of it was used for a woman's dormitory. We owe these three splendid additions to the facilities of the Laboratory, as I need hardly say, to the continued generosity, and thoughtfulness in the matter of all Laboratory needs, of the President of the Board of Trustees, Mr. Crane. Other improvements during the year were as follows: The steamer *Cayadetta* was thoroughly overhauled and her deck raised 18 inches at a cost of over $2,000, so that she is a much stauncher and more seaworthy boat than ever before. The filling in of the Laboratory harbor frontage, including the Yacht Club, has been completed; this will be graded and planted in the spring and the building moved to the east end of the frontage away from the center of the new laboratory building. Very considerable additions were made to the kitchen and laundry of the mess, power machinery electrically operated being installed for all the major operations, such as dish-washing, washing and drying of laundry, freezing ice-cream, etc. The work has thus been greatly lightened and facilitated. The Laboratory also transferred a 40-foot strip at the extreme north and west end of our Eel pond frontage to the town of Falmouth for the purposes of a boat landing, which has now been built at a cost of about $1,500, equally divided between the town and Laboratory. This was in pursuance of an agreement entered into with the town at the time of the construction of the drawbridge over the Eel pond entrance. It is therefore now possible to use the Eel pond as a harbor, and to secure delivery of supplies by this back entrance to the Laboratory property.

With all of the enlargements and additions of the past few years the Laboratory is still badly crowded during the height of the season. This applies to a certain extent to the actual accommodations for workers in the laboratory buildings, but more particularly to the housing accommodations in the village. We are planning to meet the former condition by restricting numbers in the larger classes; applications for admission will be received up to May, and appointments then made in accordance with the number of working places; if places are still available after such assignments later applicants can be admitted. It is probable that by means of various adjustments we can continue to care for all qualified investigators.

The housing accommodations in the village, however, raise a more serious question. They are entirely inadequate, and there is a consequent tendency for the prices of rooms to advance, which results in discouraging attendance. Investigators who wish to carry on regular work at the Laboratory are unable to rent houses at reasonable rates for the accommodation of their families. Conditions have become increasingly discouraging in these respects for the past several years. Moreover, land is almost unavailable for those families who wish to solve the living problem by building. It is undoubted that these conditions will exercise an inhibiting influence on the work of the Laboratory in the future, and one of the most important problems before us is to devise some means of counteracting them.

As an organization we have laid the principle of coöperation at our foundation, and we have attempted to build it into every one of our activities. Our Board of Trustees is representative of the institutions of learning of the country; our corporation represents the workers in biology on the broadest lines we can secure; our staff of instructors is widely drawn from different institutions; our students and investigators come from all parts of the accessible territory, and some from abroad; we ask only that they have a fit preparation and exhibit the spirit of scholarship. We are not tied down to any one institution or small section of the country. In past years we have considered these principles worth struggling for, and we have more than once sacrificed security to freedom of action. Our recent years have been free

and comfortable. We must not allow ourselves to forget that the principles for which we stand are never entirely won; and I appeal therefore to every member of the Board of Trustees and to all members of the corporation not to allow a sense of security to dull the edge of devotion, to keep the interests of the Laboratory at heart and to work for their support in all possible ways.

1. THE STAFF

1915

FRANK R. LILLIE, Director,
Professor of Embryology, and Chairman of the Department of Zoölogy, The University of Chicago.

GILMAN A. DREW, Assistant Director,
Marine Biological Laboratory.

ZOÖLOGY

I. INVESTIGATION

Gary N. Calkins.....Professor of Protozoölogy, Columbia University.
E. G. Conklin.....Professor of Zoölogy, Princeton University.
Gilman A. Drew.....Assistant Director, Marine Biological Laboratory.
George Lefevre.....Professor of Zoölogy, The University of Missouri.
Frank R. Lillie.....Professor of Embryology, The University of Chicago.
C. E. McClung.....Professor of Zoölogy, University of Pennsylvania.
T. H. Morgan.....Professor of Experimental Zoölogy, Columbia University.
E. B. Wilson.....Professor of Zoölogy, Columbia University.

II. INSTRUCTION

Caswell Grave.....Associate Professor of Zoölogy, Johns Hopkins University.
W. C. Allee.....Assistant Professor of Zoölogy, University of Oklahoma.

GEORGE A. BAITSELL.....Instructor in Biology, Yale University.
RAYMOND BINFORD.....Professor of Biology, Earlham College.
E. J. LUND.....Instructor in Protozoölogy, University of Pennsylvania.
T. S. PAINTER.....Instructor in Biology, Yale University.

EMBRYOLOGY

I. INVESTIGATION (see Zoölogy)

II. INSTRUCTION

WILLIAM E. KELLICOTT...Professor of Biology, Goucher College.
ROBERT A. BUDINGTON....Professor of Zoölogy, Oberlin College. (Absent in 1915.)
J. F. ABBOTT.....Professor of Zoölogy, Washington University.
CHARLES PACKARD.....Instructor in Zoölogy, Columbia University.
CHARLES C. ROGERS.....Professor of Zoölogy, Oberlin College.

PHYSIOLOGY

I. INVESTIGATION

ALBERT P. MATHEWS.....Professor of Physiological Chemistry, The University of Chicago.
RALPH S. LILLIE.....Professor of Biology, Clark University.
HAROLD C. BRADLEY...Assistant Professor of Physiological Chemistry, University of Wisconsin.
SHIRO TASHIRO.....Instructor in Physiological Chemistry, The University of Chicago.

II. INSTRUCTION

RALPH S. LILLIE.....Professor of Biology, Clark University.
WALTER E. GARREY.....Associate Professor of Physiology, Washington University Medical School.
FRANK P. KNOWLTON.....Professor of Physiology, Syracuse University.
EDWARD B. MEIGS.....Associate in Physiology, Wistar Institute of Anatomy and Biology.

PHILOSOPHICAL ASPECTS OF BIOLOGY AND ALLIED SCIENCES

LECTURES

EDWARD G. SPAULDING....Professor of Philosophy, Princeton University.

BOTANY

GEORGE T. MOORE.....Director, Missouri Botanical Garden and Professor of Botany, Washington University.

B. M. DUGGAR.....Physiologist, Missouri Botanical Garden and Professor of Plant Physiology, Washington University. (Absent in 1915.)

IVEY F. LEWIS.....Professor of Botany, University of Missouri.

R. H. COLLEY.....Instructor in Botany, Dartmouth College.

A. R. DAVIS.....Lackland Research Fellow, Missouri Botanical Garden.

W. H. WESTON.....Graduate Student, Harvard University.

LIBRARY

H. McE. KNOWER.....Professor of Anatomy, University of Cincinnati, Librarian.

MARY E. SCOTT.....Assistant Librarian.

Chemical Supplies

OLIVER S. STRONG.....Instructor in Anatomy, College of Physicians and Surgeons, New York City, Chemist.

Supply Department

G. M. GRAY.....Curator.

JOHN J. VEEDER.....Captain.

E. M. LEWIS.....Engineer.

A. W. LEATHERS.....Collector.

A. M. HILTON.....Collector.

F. G. GUSTAFSON.....Collector in Botany.

EDNA E. WELLS.....Clerk.

F. M. MACNAUGHT.....Business Assistant.

HERBERT A. HILTON.....Superintendent of Buildings and Grounds.

2. INVESTIGATORS AND STUDENTS

1915

A. ZOÖLOGY

Independent Investigators

Abbott, James F., Professor of Zoölogy, Washington University.
Addison, William H. F., Assistant Professor of Normal Histology and Embryology, University of Pennsylvania.
Allee, Warder C., Professor of Biology, Lake Forest College.
Allen, Ezra, Professor of Biology, Philadelphia School of Pedagogy.
Baitsell, George A., Instructor in Biology, Yale University.
Beckwith, Cora J., Associate Professor of Zoölogy, Vassar College.
Binford, Raymond, Professor of Zoölogy, Earlham College, Richmond.
Boring, Alice M., Associate Professor of Zoölogy, University of Maine.
Browne, Ethel N., 510 Park Ave., Baltimore, Md.
Calkins, Gary N., Professor of Protozoölogy, Columbia University.
Casteel, Dana B., Associate Professor of Zoölogy, University of Texas.
Chidester, Floyd E., Associate Professor of Zoölogy, Rutgers College.
Child, C. M., Associate Professor of Zoölogy, University of Chicago.
Clapp, Cornelia M., Professor of Zoölogy, Mt. Holyoke College.
Clark, Eleanor L., Columbia, Mo.
Clark, Eliot R., Professor of Anatomy, University of Missouri.
Conklin, Edwin G., Professor of Biology, Princeton University.
Copeland, Manton, Professor of Biology, Bowdoin College.
Cowdry, E. V., Associate in Anatomy, Johns Hopkins Medical School.
Cowdry, N. H., Johns Hopkins Medical School, Baltimore, Md.
Crampton, H. E., Professor of Zoölogy, Barnard College, Columbia Univ.
Danchakoff, Wera, Woods Hole, Mass.
Dexter, John S., Professor of Biology, University of Saskatchewan.
Donaldson, H. H., Wistar Institute of Anatomy and Biology.
Drew, Gilman A., Assistant Director, Marine Biological Laboratory.
Dunn, Elizabeth H., Woods Hole, Mass.
Gee, Wilson, Professor of Biology, Emory University, Oxford, Ga.
Goldfarb, A. J., Professor of Biology, College of the City of New York.
Goldschmidt, Richard, Member of Kaiser Wilhelm Institut Für Biologie.
Grave, Caswell, Associate Professor of Zoölogy, Johns Hopkins University.
Gregory, Emily R., Professor of Biology, University of Akron.
Gregory, Louise H., Instructor in Zoölogy, Barnard College.
Gross, Alfred O., Bowdoin College, Brunswick, Maine.
Harman, Mary T., Assistant Professor of Zoölogy, Kansas State Agricultural College.
Harvey, Basil C. H., Associate Professor of Anatomy, University of Chicago.
Heilbrunn, Lewis V., Associate in Embryology, University of Chicago.
Hegner, Robert W., Assistant Professor of Zoölogy, University of Michigan.
Hogue, Mary J., Instructor in Zoölogy, Wellesley College.
Jacobs, Merkel H., Assistant Professor of Zoölogy, University of Pennsylvania.

Kellicott, William E., Professor of Biology, Goucher College.
Knower, Henry McE., Professor of Anatomy, University of Cincinnati.
Lane, Henry H., Professor of Zoölogy, State University of Oklahoma.
Lefevre, George, Professor of Zoölogy, University of Missouri.
Lewis, Margaret R., Carnegie Institution.
Lewis, Warren H., Professor of Physiological Anatomy, Johns Hopkins Medical School.
Lillie, Frank R., Professor of Embryology, University of Chicago.
Lund, Elmer J., Assistant Professor of Zoölogy, University of Minnesota.
Malone, Edward F., Associate Professor of Anatomy, University of Cincinnati.
Macklin, Charles C., Johns Hopkins Medical School, Baltimore, Md.
McClung, Clarence E., Director of Zoölogical Laboratory, University of Pennsylvania.
Morgan, T. H., Professor of Experimental Zoölogy, Columbia University.
Morgan, Anna H., Associate Professor of Zoölogy, Mt. Holyoke College.
Morris, Margaret, Fellow in Zoölogy, Yale University.
Packard, Charles, Instructor in Zoölogy, Columbia University.
Painter, Theophilus S., Instructor in Biology, Yale University.
Pappenheimer, Alwin M., Assistant Professor of Pathology, Columbia Univ.
Patterson, John T., Professor of Zoölogy, University of Texas.
Richards, A., Instructor in Zoölogy, University of Texas.
Robertson, W. Rees B., Assistant Professor of Zoölogy, University of Kansas.
Spaeth, Reynold A., Instructor in Biology, Yale University.
Spaulding, E. G., Professor of Philosophy, Princeton University.
Stockard, Charles R., Professor of Anatomy, Cornell Medical College.
Streeter, George L., Research Associate, Carnegie Institution of Washington.
Strong, Oliver S., Instructor in Anatomy, Columbia University.
Sturtevant, Alfred H., Columbia University, New York City.
Tennent, David H., Professor of Biology, Bryn Mawr College.
Welch, Paul S., Assistant Professor of Entomology, Kansas State Agricultural College.
Woodward, Alvalyn E., Fellow in Zoölogy, University of Michigan.
Zeleny, Charles, Associate Professor of Zoölogy, University of Illinois.

Beginning Investigators

Adkins, W. S., Graduate Student, Columbia University.
Altenburg, Edgar, Assistant in Botany, Columbia University.
Binkley, Lelia T., University of Texas.
Bridges, Calvin B., Columbia University.
Cohn, Edwin J., University of Chicago.
Collett, Mary E., Instructor in Biology, Carnegie Institution of Technology.
Fish, J. Burton, Teacher of Biology, Boys' High School, Brooklyn.
Freiberg, Henry B., University of Cincinnati.
Globus, Joseph H., Assistant in Anatomy, Cornell Medical College.
Goldman, Agnes, Cornell University.
Goodrich, H. B., Union College, Schnectady.
Gould, Harley N., Fellow in Biology, Princeton University.
Hance, Robert T., Fellow in Zoölogy, University of Pennsylvania.
Hastings, George T., Teacher of Biology, DeWitt Clinton High School, Yonkers, N. Y.

Hayden, Margaret A., Instructor in Biology, Carnegie Institute of Technology.
Holt, Caroline M., Graduate Student, University of Pennsylvania.
Hickernell, Louis M., Instructor in Zoölogy, Syracuse University.
Hoy, William E., Jr., Research Student, Princeton University.
Johnson, Sydney E., Fellow in Zoölogy, Northwestern University.
Lynch, Clara J., Instructor, Smith College.
Marks, Bernice, 4 East 94th St., New York City.
Medes, Grace, Graduate Student, Bryn Mawr College.
Minoura, Tadachika, Zoölogy Department, University of Chicago.
Moore, Carl R., University of Chicago.
Osterud, Hjalmar L., Instructor in Zoölogy, University of Washington
Phillips, Ruth L., Associate Professor of Biology, Western College.
Reagan, Franklin T., Fellow in Biology, Princeton University.
Root, Francis M., Johns Hopkins University.
Safir, Shelley R., Teacher in New York High School, New York City.
Vanneman, Aimée S., Technician, University of Texas.
Ware, Clara C., Graduate Student, Columbia University.
Weinstein, Alexander, Columbia University.
Wheat, Frank M., Columbia University.
Whiting, Phineas W., University of Pennsylvania.
White, Edith G., Columbia University.
Wilkins, Lawson, Johns Hopkins University.

B. PHYSIOLOGY

Independent Investigators

Bogacki, Kamil J., Physician, Western Reserve University.
Chambers, Robert, Jr., Assistant Professor of Histology and Comparative Anatomy, University of Cincinnati.
Edwards, Dayton J., Instructor in Physiology, College of the City of New York.
Garrey, Walter E., Associate Professor of Physiology, Washington University.
Hyde, Ida H., Professor of Physiology, University of Kansas.
Just, Ernest E., Professor of Physiology, Howard University.
Kingsbury, Francis B., Instructor in Physiological Chemistry, University of Minnesota.
Knowlton, Frank P., Professor of Physiology, Syracuse University.
Lillie, Ralph S., Professor of Biology, Clark University.
Loeb, Jacques, Head of Department of Experimental Biology, Rockefeller Institute for Medical Research.
Mathews, Albert P., Professor of Physiological Chemistry, University of Chicago.
Meigs, Edward B., 1722 H. Street, N. W., Washington, D. C.
Moore, Arthur R., Associate Professor of Physiology, Bryn Mawr College.
Rogers, Charles G., Professor of Comparative Physiology, Oberlin College.
Scott, Ernest L., Associate in Physiology, Columbia University.
Tashiro, Shiro, Instructor in Physiological Chemistry, University of Chicago.
Waller, John C., Graduate Student, University of Chicago.
Warren, Howard C., Professor of Psychology, Princeton University.
Wasteneys, Hardolph, Associate in Experimental Biology, Rockefeller Institute for Medical Research.
Werber, Ernest L. Sessel Research Fellow, Yale University.

Beginning Investigators

Atwood, W. G., Dartmouth College.
Chamberlain, Mary M., Bryn Mawr College.
Levy, Augustus, University of Chicago.
Cattell, McKeen, Harvard Medical School.

C. BOTANY

Independent Investigators

Blakeslee, A. F., Professor of Botany and Genetics, Connecticut Agricultural College.
Colley, R. H., Instructor in Botany, Dartmouth College.
Hibbard, R. Percival, Plant Physiologist, Michigan Agricultural College.
Lewis, Ivey F., Professor of Botany, University of Missouri.
Moore, George T., Director, Missouri Botanical Garden, St. Louis.
Weston, William H., Jr., Instructor in Botany, Harvard University.

Beginning Investigators

Cobb, Ruth, Falls Church, Virginia.
Smith, Pearl M., Assistant in Botany, University of Wisconsin.

STUDENTS

1915

1. ZOÖLOGY

Adams, A. Elizabeth, Instructor in Zoölogy, Mount Holyoke College.
Andrus, Edwin C., Student, Oberlin College, Oberlin, Ohio.
Andrus, William D. W., Student, Oberlin College, Oberlin, Ohio.
Bain, Therese S., Vassar College, Poughkeepsie, N. Y.
Baldwin, Imogen, Student, Mt. Holyoke College, So. Hadley, Mass.
Bell, Charles E., Student, Ursinus College.
Blanchard, Nellie P., Head of Biological Dept., Hood College.
Cattell, Owen, Garrison-on-Hudson.
Cattell, Psyche, Student, Sargent, Cambridge, Mass.
Charlton, Harry H., Assistant in Zoölogy, Yale University.
Cocks, Edmund, Graduate Student, Columbia University, New York City.
Cobb, Margaret C., Barnard College, New York City, N. Y.
Cowdery, Lawrence T., Student, Oberlin College, Oberlin, Ohio.
Cross, Howard B., Instructor, Oklahoma University, Norman, Okla.
Dibell, Mabel E., Instructor of Biology, Western College.
Ehrenfeld, Frederick, Assistant Professor of Geology, University of Pennsylvania.
Gilbert, Marion, Student, Oberlin College, Oberlin, Ohio.
Greisheimer, Esther M., Clark University, Worcester, Mass.
Harvey, Helen F., Oberlin College, Oberlin, Ohio.
Harris, Coleman J., Lewisburg, Pa.

Hayden, Ruth, Goucher College, Baltimore, Md.
Hea, Emily M., Teacher in High School, Morristown, N. J.
Heeman, Harriet M., Student, Oberlin College, Oberlin, Ohio.
Howe, Marion G., Mt. Holyoke College, So. Hadley, Mass.
Hughes, Dorothea M., Simmons College, Boston, Mass.
Hulst, Myra M. Vassar College, Poughkeepsie, N. Y.
Irvine, Helen, Student, Mt. Holyoke College, So. Hadley, Mass.
Josephs, H. W., Assistant in Chemistry, Harvard University, Cambridge.
Kostir, Wencel J., Instructor, Ohio State University, Columbus, Ohio.
Ludwig, Albert P., Student, Oberlin College, Oberlin, Ohio.
Marinus, Carleton J., Syracuse University, Syracuse, N. Y.
Mahr, Ernst F., Jr., Syracuse University, Syracuse, N. Y.
McGrath, Jule G., Laboratory Assistant, Hunter College, New York City.
Moses, Gertrude, 2002 Bolton St., Baltimore, Md.
Oudesluys, Hortense, Teacher, Western High School, Baltimore, Md.
Pennypacker, Frances W., Sweet Briar College.
Ripple, Clarence V., Student, University of Pennsylvania, Philadelphia.
Rogers, Mildred, Student, Goucher College, Baltimore, Md.
Runyon, Paul M., Student, Princeton University, Princeton, N. J.
Sanford, Eldon W., Graduate Student, Yale University, New Haven, Conn.
Sheldon, Paul B., Student, Oberlin College, Oberlin, Ohio.
Smith, Sue F., Carnegie Institute of Technology.
Smith, Christianna, Assistant in Zoölogy Laboratory, Mt. Holyoke College, So. Hadley, Mass.
Smith, Inez C., Student, Mt. Holyoke College, So. Hadley, Mass.
Spence, Margaret, Instructor La Crosse State Normal School, La Crosse.
Wilder, Katherine, Science Assistant, The Newton High School.
Wright, Helen G., Student, Mt. Holyoke College, So. Hadley, Mass.

2. EMBRYOLOGY

Ashman, Richard, Rutgers College, New Brunswick, N. J.
Allard, Ann D., Teacher, So. Boston, Mass.
Blanchard, Frank H., Instructor, Tufts College, Mass.
Blau, Julia E., Princeton, N. J.
Cahn, Alvin R., Assistant in Zoölogy, University of Wisconsin, Madison, Wis.
Carroll, Mitchel, 617 So. 16th St., Philadelphia, Pa.
Closson, James H., Student, Princeton University, Princeton, N. J.
Cobb, Dorothy, Student, Radcliffe College, Cambridge, Mass.
Cutter, Elizabeth, Assistant in Zoölogy, Vassar College.
Farnum, Alice R., Student, Smith College, Northampton, Mass.
Fish, Gordon T., Assistant, Yale University, New Haven, Conn.
Hamlin, Howard E., Student, Harvard University, Cambridge, Mass.
Henry, Edna M., Student, Barnard College, New York City, N. Y.
Herrick, Joseph C., Professor of Biology, St. Joseph's Seminary, Yonkers, N. Y.
Hufford, Clarence E., Student, Oberlin College, Oberlin, Ohio.
Hurlin, Ralph G., Instructor, Clark College.
Jamieson, Janet P., Student, University of Pennsylvania.
Kakiuchi, Samuro, Yale University, New Haven, Conn.
Lewis, Elsie M., Student, Oberlin College, Oberlin, Ohio.

Macy, Cora F., Student, Syracuse University, Syracuse, N. Y.
Mallard, Agnes K., Teacher, 1658 Columbia Road, So. Boston, Mass.
Myers, Mae L., Associate Professor of Anatomy, Woman's Medical College of Pennsylvania.
Millikan, Frances, Student, Smith College, Northampton, Mass.
Montgomery, Priscilla B., 105 So. 41 St., Philadelphia, Pa.
Parmenter, Charles L., Assistant Professor, University of Wisconsin.
Patten, Mary W., Goucher College, Baltimore, Md.
Richards, Lyman G., 259 Prospect St., Fall River, Mass.
Richards, George L., 124 Franklin St., Fall River, Mass.
Sherwood, Helen L., Student, Vassar College, Poughkeepsie, N. Y.
Stocking, Ruth J., Professor of Biology, Agnes Scott College, Decatur.
Stocking, Besse E., Student, Goucher College, Baltimore, Md.
Straus, Aubrey H., Associate Professor of Bacteriology, Medical College of Virginia, Richmond, Va.
Thomas, Anna M., Student, Carnegie Institute of Technology.
Webster, Leslie T., Amherst College, Amherst, Mass.
Williams, G. Huntington, Graduate Student, Johns Hopkins Medical School.
Williams, James W., Lincoln St., New Haven, Conn.
Winslow, Mina L., Graduate Student, University of Michigan.

3. PHYSIOLOGY

Adler, Francis H., University of Pennsylvania, Philadelphia, Pa.
Bensing, LeRue P., Research Assistant, Research Commission of Natural Dental Association, Cleveland, Ohio.
Bodine, Joseph H., University of Pennsylvania, Philadelphia, Pa.
Fenn, Wallace O., Harvard University, Cambridge, Mass.
Eggstein, A. A., Instructor in Pathology, Vanderbilt College.
Holt, Emmett, 14 West 55th St., New York City, N. Y.
Hughes, Walter S., Massachusetts Institute of Technology.
Loeb, Robert F., Student, University of Chicago, Chicago, Ill.
Lundgren, C. Albert, Assistant in Biology, Clark University.
Metcalf, John T., Instructor in Psychology, Princeton University.
Minnich, Dwight E., Harvard University, Cambridge, Mass.
Price, Weston A., Chairman, The Research Commission of the National Dental Association, Cleveland, Ohio.
Reeves, Prentice, Assistant in Experimental Psychology, Princeton Univ.
Schneider, Peter A., Instructor in Zoölogy, University of Vermont.
Thompson, Martha, Teacher of Biology, Bay Ridge High School, New York City, N. Y.

4. BOTANY

McConnell, Louise J., 341 Rector St., Perth Amboy, N. J.
Mong, Grace E., Student, Oberlin College, Oberlin, Ohio.
Oak, Dorothy, Student, Barnard College, New York City, N. Y.
Severy, J. Warren, Student, Oberlin College, Oberlin, Ohio.
Yasui, Kono, Student, Radcliffe College, Cambridge, Mass.
Young, Anna R., Student, Smith College, Northampton, Mass.

3. TABULAR VIEW OF ATTENDANCE

	1911	1912	1913	1914	1915
INVESTIGATORS—Total	82	93	122	129	137
Independent:					
Zoölogy	42	44	58	62	69
Physiology	18	14	17	22	20
Botany	8	10	11	10	6
Under Instruction:					
Zoölogy	12	21	21	31	36
Physiology	2	2	7	1	4
Botany		2	7	3	2
STUDENTS—Total	65	67	69	89	105
Zoölogy	26	24	33	43	47
Embryology	20	15	22	21	37
Physiology	6	11	8	10	15
Botany	13	17	7	15	6
TOTAL ATTENDANCE	147	160	191	218	242
INSTITUTIONS REPRESENTED—					
Total		57	80	77	79
By investigators	37	43	50	51	59
By students	31	36	41	47	42
SCHOOLS AND ACADEMIES REPRESENTED.					
By investigators	3	2	3	1	3
By students	9	1	6	5	9

4. SUBSCRIBING INSTITUTIONS—1915

AMHERST COLLEGE
BARNARD COLLEGE
BOWDOIN COLLEGE
BRYN MAWR COLLEGE
CARNEGIE INSTITUTE OF TECHNOLOGY
CARNEGIE INSTITUTION, WASHINGTON
CLARK UNIVERSITY
COLUMBIA UNIVERSITY
DARTMOUTH COLLEGE
ELSE SERINGHAUS SCHOLARSHIP, HUNTER COLLEGE, NEW YORK CITY
GOUCHER COLLEGE

Harvard University
Johns Hopkins University
Kansas State Agricultural College
Lucretia Crocker Scholarships
Mount Holyoke College
Northwestern University
Oberlin College
Princeton University, Dept. of Biology
Princeton University, Dept. of Psychology
Radcliffe College
Rockefeller Institute for Medical Research
Rutgers College
Smith College
Syracuse University
University of Chicagq
University of Illinois
University of Kansas
University of Michigan
University of Pennsylvania
University of Texas
University of Wisconsin
Vassar College
Wellesley College
Western College
Wistar Institute
Yale University

5. EVENING LECTURES, 1915

Friday, July 2,
Prof. C. R. Stockard.. "Experimental Production of Racial Degeneracy by Alcohol Poisoning."

Tuesday, July 6,
Prof. C. H. Parker.... "The Seals of the Pribiloff Islands."

Friday, July 9,
Prof. G. L. Streeter.. "Some Experimental Studies on the Development of the Membranous Labyrinth in the Tadpole."

Tuesday, July 13,
Prof. E. G. Conklin... "Effects of Centrifugal Force on the Structure and Development of the Egg."

Friday, July 16,
PROF. R. S. LILLIE . . . . . "The Nature of Intelligent and Purposive Action from a Physiological Point of View."
Monday, July 19,
PROF. SIMON FLEXNER . . "The Control of Infection as Affected by Variation Among Parasitic Microörganisms."
Saturday, July 24,
PROF. G. N. CALKINS . . . "Protozoa and the Cancer Problem."
Friday, July 30,
PROF. GEORGE SHULL . . . "Inheritance of Sex in *Lychnis*."
Tuesday, Aug. 3,
PROF. C. B. DAVENPORT "Heredity of Criminality."
Friday, Aug. 6.
DR. MARTIN EDWARDS . . "The Story of Bubonic Plague."
Tuesday, Aug. 10,
DR. ALFRED G. MAYER. "The Rôle of Adsorption in Nerve Conduction."

6. MEMBERS OF THE CORPORATION

1. LIFE MEMBERS

ALLIS, MR. E. P., JR., Palais Carnoles, Menton, France.
ANDREWS, MRS. GWENDOLEN FOULKE, Baltimore, Md.
BILLINGS, MR. R. C., 66 Franklin St., Boston, Mass.
CAREY, MR. ARTHUR ASTOR, Fayerweather St., Boston, Mass.
CLARKE, PROF. S. F., Williams College, Williamstown, Mass.
CONKLIN, PROF. EDWIN G., Princeton University, Princeton, N. J.
CRANE, MR. C. R., Woods Hole, Mass.
DAVIS, MAJOR HENRY M., Syracuse, N. Y.
EVANS, MRS. GLENDOWER, 12 Otis Place, Boston, Mass.
FARLOW, PROF. W. G., Harvard University, Cambridge, Mass.
FAY, MISS S. B., 88 Mt. Vernon St., Boston, Mass.
FOLSOM, MISS AMY, 88 Marlboro St., Boston, Mass.
FOOT, MISS KATHERINE, 80 Madison Ave., New York City, N. Y.
GARDINER, MRS. E. G., Woods Hole, Mass.
GARDINER, MISS EUGENIA, 15 W. Cedar St., Boston, Mass.
HANNAMAN, MR. CHARLES E., 103 1st St., Troy, N. Y.

HARRISON, EX-PROVOST C. C., University of Pennsylvania, Philadelphia, Pa.
JACKSON, MISS M. C., 88 Marlboro St., Boston, Mass.
JACKSON, MR. CHAS. C., 24 Congress St., Boston, Mass.
KENNEDY, MR. GEO. G., 284 Warren St., Roxbury, Mass.
KIDDER, MR. C. G., 27 William St., New York City, N. Y.
KIDDER, MR. NATHANIEL T., Milton, Mass.
KING, MR. CHAS. A.
LEE, MRS. FREDERIC S., 279 Madison Ave., New York City, N. Y.
LOWELL, MR. A. LAWRENCE, 171 Marlboro St., Boston, Mass.
MARRS, MRS. LAURA NORCROSS, 9 Commonwealth Ave., Boston, Mass.
MASON, MR. E. F., 1 Walnut St., Boston, Mass.
MASON, MISS IDA M., 1 Walnut St. Boston, Mass.
MEANS, MR. JAMES HOWARD, 196 Beacon St., Boston, Mass.
MERRIMAN, MRS. DANIEL, Worcester, Mass.
MINNS, MISS SUSAN, 14 Louisburg Square, Boston, Mass.
MINNS, MR. THOMAS, 14 Louisburg Square, Boston, Mass.
MIXTER, MISS M. C., 241 Marlboro St., Boston, Mass.
MORGAN, MR. J. PIERPONT, JR., Wall and Broad Sts., New York City, N. Y.
MORGAN, PROF. T. H., Columbia University, New York City, N. Y.
MORGAN, MRS. T. H., New York City, N. Y.
NOYES, MISS EVA J., 28 South Willow St., Montclair, N. J.
NUNN, MR. LUCIAN L. Telluride, Colo.
OSBORN, PROF. HENRY F., American Museum of Natural History, New York.
PHILLIPS, DR. JOHN C., Windy Knob, Newham, Mass.
PHILLIPS, MRS. JOHN C., Windy Knob, Newham, Mass.
PORTER, DR. H. C., University of Pennsylvania, Philadelphia, Pa.
PULSIFER, MR. W. H., Newton Center, Mass.
ROGERS, MISS A. P., 5 Joy St., Boston, Mass.
SEARS, DR. HENRY F., 420 Beacon St., Boston, Mass.
SHEDD, MR. E. A.,
SMITH, MRS. C. C., 286 Marlboro St., Boston, Mass.
STROBELL, MISS E. C., 80 Madison Ave., New York City, N. Y.
THORNDIKE, DR. EDWARD L., Teachers College, Columbia University, New York City, N. Y.

TRELEASE, PROF. WILLIAM, University of Illinois, Champaign, Ill.
WARE, MISS MARY L., 41 Brimmer St., Boston, Mass.
WARREN, MRS. S. D., 67 Mt. Vernon St., Boston, Mass.
WHITNEY, MR. HENRY M., Brookline, Mass.
WILLCOX, MISS MARY A., Wellesley College, Wellesley, Mass.
WILMARTH, MRS. H. D., Elliott St., Jamaica Plain, Mass.
WILLIAMS, MRS. ANNA P., 505 Beacon St., Boston, Mass.
WILSON, DR. E. B., Columbia University, New York City, N. Y.
WILSON, PROF. W. P., Philadelphia Museum, Philadelphia, Pa.

2. MEMBERS, JANUARY, 1916

ABBOTT, PROF. J. F., Washington University, St. Louis, Mo.
ABBOTT, MISS MARGARET B., The Bennett School, Milbrook, N. Y.
ADDISON, DR. W. H. F., University of Pennsylvania, Medical School, Philadelphia, Pa.
ADKINS, MR. W. S., Texas Christian University, Fort Worth, Texas.
ALLEE, DR. W. C., Lake Forest College, Lake Forest, Ill.
ALLEN, PROF. EZRA, 125 Thompson Ave., Ardmore, Pa.
ALLYN, MISS HARRIET M., Hackett Medical College, Canton, China.
ALSBURG, DR. C. S., U. S. Dept. of Agriculture, Washington, D. C.
BAITSELL, DR. GEORGE A., Sheffield Scientific School, Yale University, New Haven, Conn.
BAKER, DR. E. H., 154 W. Randolph St., Chicago, Ill.
BANCROFT, DR. F. W., Aloha Farm, Concord, California.
BARDEEN, PROF. C. R., University of Wisconsin, Madison, Wis.
BECKWITH, MISS CORA J., Vassar College, Poughkeepsie, N. Y.
BEHRE, MISS ELINOR H., University of Chicago, Chicago, Ill.
BEYER, DR. H. G., Stoneleigh Court, Washington, D. C.
BIGELOW, PROF. M. A., Teachers College, Columbia University, New York City, N. Y.
BIGELOW, PROF. R. P., Mass. Institute of Technology, Boston, Mass.
BINFORD, DR. RAYMOND, Earlham College, Richmond, Ind.

Binkley, Miss Lelia T., University of Texas, Austin, Texas.
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AMITOSIS IN CELLS GROWING IN VITRO.

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(3 PLATES, 27 FIGURES.)

CONTENTS

INTRODUCTION.

During recent years the conviction among cytologists has become more and more strongly intrenched that the problem of amitosis can not be satisfactorily solved by investigations based alone upon the study of non-living tissue, but that its successful conquest must rely principally upon the correct interpretation of the succession of morphological and physiological changes revealed by prolonged observation of the living cell, under normal and artificially varied conditions. Yet until the advent of the tissue culture method, workers in biology might well have despaired of ever being able to attack the question in this way. It is fortunate, therefore, that the technique of tissue cultivation has been so perfected that even the minute structural details of the living cell may be readily observed, and that the inspection may be continued for hours at a time; fortunate, too, that configurations which may be interpreted as stages in the process of direct division, such as dumb-bell-shaped nuclei apparently undergoing constriction, and bipartite nuclei, are not infrequently found in tissue cultures, and, furthermore, that fixed and stained preparations, to assist in the interpretation of the appearances

presented by the living cells, are easily made from such cultures.

These favorable circumstances plainly indicated the course to be followed in an attempt to gain some knowledge of direct cell division, and accordingly continuous observations of living cells, and supplemental studies of fixed and stained cells of the same type, were carried out.[1]

Method

The cells upon which observations were made were growing at a temperature of 39° to 40° Cent. from the tissue of embryo chicks from two to ten days old. Cultures were prepared by the method of Lewis and Lewis ('15), Locke solution being used as a medium. Activation of the growth was accomplished by the addition to the medium of a small quantity of autogenous embryo extract or bouillon. Thus the medium contained, besides the various salts, small quantities of dextrose (.25 to 1 per cent.) and protein. To offset the concentration due to evaporation during planting and in the moist chamber it was found to be of advantage to dilute the medium by adding 20 to 25 per cent. of freshly distilled sterile water. Heart tissue was most frequently used, and gave growths which were most serviceable for observation during the second twenty-four hour period.

By arranging the microscope within the incubator where the tissues were cultivated it was not necessary to expose them to a changed temperature during observation. A ray screen of copper sulphate solution was found to be advantageous when artificial light was used. Evaporation of the drop, with condensation about the walls of the moist chamber, was lessened by placing a small drop of distilled water in the cavity of the depressed slide, and by eliminating air currents from the vicinity of the culture.

Light seemed to have a deleterious effect upon the living cultures, so that lengthy continuous observation was not found to be practicable. Accordingly inspections were made as short

[1] The procedure of studying amitosis by the tissue culture method was suggested by M. R. and W. H. Lewis, and I desire to record my appreciation of their kind assistance and the use of their large collection of fixed and stained cultures, which was utilized in the investigation. To Prof. F. R. Lillie I also am indebted for his courtesy in placing a room at the Marine Biological Laboratory at my disposal, where some of the work was carried on.

as possible, and the light immediately turned off. The difficulties attending direct and prolonged observation of the living cell are not inconsiderable, for the eye must become accustomed to distinguish minute structures through the contrast afforded by varying grades of refractivity. At first only the most refractive bodies are discernible, standing out as bright points or lines, but gradually the less obvious structures, as mitochondria, and amœboid cell processes, come into view. Migration, in many of the cells, is quite active, and hence it is necessary to make observations frequently to prevent the cell from wandering away from the field of vision and becoming lost. The extreme sensitivity of the cell to light and heat, and to changes in osmotic pressure of the media from evaporation within the moist chamber, is responsible for the untimely termination of many observations, and this difficulty becomes the more important when it is realized that the study of a single cell must cover many hours to be complete.

It was at first planned to select a single living normal cell and observe it at frequent intervals over a long period in the hope that eventually a cell would be found which would divide by amitosis. This course, however, did not prove to be practicable on account of the infrequency with which amitosis, even of the nucleus alone, is met with. In a series of 20 fixed and stained growths from the heart of the embryo chick, in which there was a total of 41,725 cells,[1] only 50 constricted nuclei, which could be regarded as directly dividing, were found (a ratio of 1 to 835), and thus the chances of the occurrence of amitotic nuclear division in a cell selected at random were but little better than one tenth of one per cent. To avoid loss of time, therefore, the plan was adopted of selecting a cell in which the amitotic process

[1] The method adopted in making counts was as follows: 20 good preparations from cultures of chick heart of various ages and stages of growth which had been fixed and stained were selected. A small square was ruled with a diamond upon a piece of glass after the method of Isaacs ('15), and this ruled glass was inserted in the ocular so that a definitely outlined field was marked off upon the tissue culture preparation on the stage. By manipulating the mechanical stage successive fields could easily be brought into view, and the cells contained in them counted; in this way all the cells in the entire new growth were counted except imperfect cells and those near the original piece, which were several layers deep and were indistinct.

appeared to have already commenced, viz., in which the nucleus showed elongation and equatorial constriction, and observing in detail the subsequent changes which it underwent.

Such a cell presents an appearance of the following general type. The nucleus is somewhat lengthened, and, in the zone equidistant from its poles there is to be seen, on one or both sides, an indentation. In this concavity is situated characteristically a body, the centrosphere (fig. 1, *c*) whose refractivity is somewhat greater than that of the surrounding cytoplasm. Its outline is indistinct, but the edge seems to be irregular with short toothlike processes. These change their shape slowly, and give the impression of being pushed out and drawn in very gradually. They are intimately related to definite refractive bodies—the mitochondria—which, often rodlike and sometimes threadlike in form, radiate from the periphery of the centrosphere. Indeed the movements of the latter (described by Lewis and Lewis '15) may be responsible for the apparent movement in the periphery of the centrosphere.

Preparations fixed with osmic acid vapor and stained with Heidenhain's iron hematoxylin (which was the method generally employed) disclosed a minute granular body, generally paired, within the centrosphere, which is recognized as the centrosome or centriole (Fig. 24).

The position of the centrosphere within the nuclear concavity or cleft has been noted by various authors, including Maximow ('08), who found the centriole-pair thus situated in cells, the nuclei of which appeared to be dividing directly, in the mesenchyme of the embryo rabbit. In the cells of tissue cultures examined the centrosphere was absent from the cleft in only two per cent. of cases, and in these exceptions it may have been originally situated as in the others.

Observations.

A number of extended observations were made upon living cells containing such elongated and constricted nuclei, and, as a general rule, the nucleus rounded out again, assuming the usual form. Thus it was demonstrated that constriction alone does not indicate that the nucleus will divide directly. Finally,

however, a cell was found in which the nucleus divided directly while being watched, and the following paragraph, extracted from the protocol written at the time, is a brief description of the process as observed. The drawings (Plate I.) were sketched free-hand from observation at fifteen-minute intervals, and afterward retouched by reference to fixed and stained cells of similar morphology.

8.45 P.M. A cell (Fig. 1), growing from a 57-hour culture of 5-day chick heart, presented an elongated nucleus with a concavity upon one side. The outline of this concavity was indistinct on account of the fact that the centrosphere (*c*) was situated very close to it.

9.00 P.M. (Fig. 2). The nucleus is now straight and the indentation is almost obliterated.

9.15 P.M. (Fig. 3). The general outline of the cell has changed, and this has been followed by change in shape of the nucleus. At first there were two nucleoli within the nucleus, the lowermost being paired, but at 9.30 (Fig. 4) the latter appears as a dumb-bell-shaped body. The nucleus is now rounded.

9.45 P.M. (Fig. 5). The nucleus is still rounded, and the nucleolar substance consists of two masses close together.

10.00 P.M. (Fig. 6). The nucleus has become elongated again. There is no apparent cleft, but the left side, against which the centrosphere is resting is not so distinct as the right. The central nucleolus now appears as a single structure and some refractive substance, resembling another nucleolus, is seen in the lowermost pole of the nucleus.

During the next two fifteen-minute intervals (Figs. 7 and 8) a shortening of the nucleus occurred, a shallow concavity to the left being noted. This concavity is deeper at 10.45 (Fig. 9) its outlines being somewhat indistinct and the nucleus has increased in length; across the middle of the nucleus is a refractive line.

11.00 P.M. (Fig. 10). The concavity is seen with difficulty, and the line across the nucleus persists. Fifteen minutes after this (Fig. 11) the elongated nucleus shows an indentation upon the right side, opposite the one upon the left, and it appears to be undergoing constriction into two parts of equal size. Between the two nuclear portions the refractive line is seen as before, and, from the appearance shown in cells fixed and stained with iron hematoxylin (Fig. 24) this is evidently a strand of mitochondria.

11.30 P.M. (Fig. 12). The nuclear sacs are apparently quite separate, and between them is the strand of mitochondria, and also part of the centrosphere, this body, still undivided, having retained its position with reference to the nuclear cleft.

Judging from the behavior of this and other elongated, bent and dumb-bell shaped nuclei it would seem that the nucleus may return to its original rounded form provided the constriction has not gone too far, but, if the degree of constriction passes a certain critical point the nuclear sacs become completely separated.

Furthermore, after the critical point is passed the division occurs very rapidly.

The study of fixed and stained specimens served to throw considerable light upon the process of direct division, for, by searching the field, transitional forms were encountered, suggesting stages in the history of the living nucleus just described. In the terminal stage of direct nuclear division, such as that shown in Fig. 24, mitochondria were found characteristically lying across the slender strand of nuclear membrane which was all that remained of the connection between the two portions of the nucleus, and the centrosphere (which is undivided in these transitional forms, and also in the end product of nuclear amitosis, the binucleate cells) is situated in the cleft dividing these parts.

This process involves a more or less equal mass division of the nucleus without chromosome formation. It results in the production of a binucleate cell, and, if the process is repeated, of a trinucleate or multinucleate cell. Direct nuclear division was not observed beyond the bipartite stage, but it seems rational to suppose that (excluding the foreign body giant cells of Lambert (1912 *a* and *b*) the giant cells of tissue cultures are formed from the successive direct splitting of the nuclear fragments which become larger by normal growth. With this latter there is apparently associated an increase in the cytoplasm also.

It was not practicable to make a complete study of amitosis upon the same cell, and the latter stages of this process, succeeding direct division of the nucleus, were studied by selecting living binucleate cells and making prolonged observations upon them. A typical case, from a 24-hour culture of 8-day chick heart, is given as follows:

11.50 A.M. The cell, which was of the characteristic connective tissue type, showed two separate nuclear sacs, whose adjacent surfaces were in close contact. One sac contained three nucleolar fragments, the other one. Fat granules were fairly abundant, and were principally congregated at the nuclear poles. Mitochondria, long and threadlike, stretched between these granules and, where the latter were abundant, the strands of mitochondria tended to arrange them in rows. Mitochondria also radiated from the single centrosphere, situated opposite the area of contact of the two nuclear sacs. The triangularly shaped cell body was connected with adjacent cells by three main processes.

12.20 P.M. A narrow interval can be seen between the nuclear parts, showing that the latter have moved apart and are quite separate. Their position also has

changed. Variation in the nucleoli is noted, there being now three in one nuclear sac and four in the other. The outline of the cell body is now quadrilateral, and this shifting of form has perhaps accounted for the rearrangement in relative position and relationship of the nuclear parts. These, at 2.30, were again in contact, but subsequently repeated the process of moving apart and coming together three times during the observation, which ended at 11.15 p.m. During this time (11½ hours) the cell was observed continuously, and underwent constant minor changes, such as that of the outline, shifting about of cytoplasmic structures, and breaking up, recombination and variation in size and shape of the nucleolar fragments. After almost twelve hours the cytoplasm showed no indication of dividing.

These observations brought out the fact that what might be mistaken for a single nucleus divided by a membrane across its equator is really two nuclear sacs pressed close together, the equal tension in the two bodies resulting in a flat membrane between them, made up of the surfaces of contact. Child (1911) describes a type of amitosis which is characterized apparently by the growth through the nucleus of a membrane or plate, the subsequent splitting of which leads to the production of two nuclear sacs quite separate one from the other. Nuclear fission of this type was not found, and appearances suggesting a process of this kind probably result from the close relationship of separate nuclear parts, similar to the condition found in the cells of tissue cultures. Partitions within the nucleus are also simulated, in these flattened cells, by long nucleoli, mitochondria or folds in the nuclear membrane.

These observations also illustrate the characteristic behavior of the nucleolar bodies, which undergo constant changes in size, shape, number and position. These bodies stain well with gentian violet when applied to the living culture. They appear as dark masses after iron hematoxylin, but if differentiation with iron alum is carried too far what was before a homogeneous mass becomes a collection of granules (Fig. 27). The nucleolus appears to be a concentrated gel of varying density, the granules representing the denser areas.

Similar observations upon other living cells were carried out, and in no case did any direct fission of the cytoplasm occur; this finding was supported by the study of fixed preparations. On the contrary the history of these binucleate cells was the same as that of mononucleate cells of the same type. It was even found that mitosis occurred in these cells, for, during the obser-

vation of a binucleate cell in a young culture the good fortune was encountered of witnessing this entire process in it. A graphic register of the successive changes is afforded by the drawings (Plate II.) which were made with the aid of a camera lucida at the times stated. The following is extracted from the protocol written at the time of examination:

11.55 A.M. A typical connective tissue binucleate cell (Fig. 13) from a 19-hour culture of 7-day chick heart was selected for observation. The two approximately equal, sharply outlined nuclear sacs are in close contact, causing a flattening of the apposed surfaces. Each nucleus contains a single, somewhat irregular nucleolus. The cell is long and narrow, and the long axis of the nucleus is parallel with that of the cell in which it is contained. A single centrosphere (*c*) is found opposite the area of contact of the two nuclear parts. Numerous fat globules and mitochondria, the latter showing characteristic movements, are seen.

12.40 P.M. (Fig. 14). The nucleus is still double, and the principal change noted is the appearance of an additional nucleolus in the lower nuclear part.

1.20 P.M. (Fig. 15). The two parts of the nucleus are distinct. Only one nucleolus is now seen in each nuclear sac.

1.50 P.M. (Fig. 16). The cell outline has become modified, the cell body being shorter, and the long axis of the nucleus has changed so that it is now almost at right angles to its former direction. The nuclear surfaces are in close contact and the upper end of the membrane formed by their approximation is indefinite in outline.

3.05 P.M. (Fig. 17). The cell body has become more fusiform, and the long axis of the nucleus has rotated through 90°. The double membrane (formed by the areas of nuclear wall in contact) dividing the nuclear portions cannot be made out except at the left side, and there indistinctly. An indefinite, refractive substance, granular in character, is scattered along the line where this membrane had been; the nucleoli are indefinite, and the uppermost one has been joined by an additional, very small, mass of the same character. The upper part of the nuclear membrane is not so distinct as heretofore.

It would appear that the change which has occurred in the part of the nuclear membrane dividing the two nuclear sacs is a part of the general change affecting the entire nuclear wall and leading to its gradual disappearance, and the process is similar to that which occurs in the early stages of mitosis.

5.05 P.M. (Fig. 18). The refractive material in the zone formerly separating the two nuclear sacs is more prominent than before; it seems to be chromatin. The nuclear membrane is faintly marked.

Half an hour later this cell is seen to become gradually smaller, and to draw in its processes. At the same time the nuclear parts become smaller. The nucleoli also undergo diminution in size. Finally the cell takes a rounded and thickened form—6.00 P.M. (Fig. 19)—and is much more refractive than the cells surrounding it: in fact it resembles a cell in the prophase of mitosis. The fat globules and mitochondria assume a wreath-like appearance about the central clear space, in which the nucleoli soon disappear. Though the main mass of the cell is almost circular there are narrow processes attached to each pole. The cell remains apparently unchanged for some time, though undoubtedly important readjustments are going on within it.

6.50 P.M. (Fig. 20). The cell is even smaller than before, and more rounded. In the clear area within it is a refractive bar, which proves to be the equatorial plate of chromosomes. Individual chromosomes cannot be distinguished, so that it is impossible to count them, but their ends may be seen, as they project towards the poles of the spindle. The chromosomes show a slow, oscillatory type of movement, slight in extent. The spindle is represented by a fairly clear area, shaped like two cones base to base, at the extremities of which the centrosomes are situated. Astral rays cannot be seen. Mitochondria and fat globules encase this central area containing the spindle like a shell. This becomes evident by focusing up and down. Though the uppermost part of the spherical cell is on a level with the flat resting cells, which are to be found about it, the lowermost part is much below this, due to the fact that the cell is thickened, and projects into the medium.

7.05 P.M. (Fig. 21). The cell has suddenly become elongated and constricted at the equator; the plate of chromosomes has evidently split, and the anaphase of mitosis is being witnessed. The constriction about the middle of the cell can be seen to be increasing, causing streaming of globules toward its extremities. At the same time small, bubble-like processes emerge from the borders of the cell, seeming to be forced out by the internal pressure of the cell body. Into these protuberances granules flow but later return into the main mass of the cytoplasm. These processes soon become flattened and extended, forming pseudopodia possessed of hyaline borders with amœboid movement. The individual chromosomes of the two masses in the expanded extremities gradually lose their distinctness and become dispersed.

7.25 P.M. (Fig. 22). The constriction is more marked and the cells are almost entirely separated. They are also becoming flattened out. In the upper daughter cell a clear space for the nucleus is appearing. The constricted zone is somewhat more highly refractive than the surrounding tissue and resembles a short thread. This probably contains part of the remains of the spindle. Nuclear details are not yet visible.

As the cell is watched nucleoli become manifest, at 7.35 P.M. two of these being seen in each daughter nucleus in a clear space, surrounded by a distinct nuclear membrane.

8.00 P.M. (Fig. 23). The daughter cells are now almost of normal size. Each nucleus has a concave side, as is usual, and in each concavity is the new centrosphere, from which the mitochondria radiate. Two nucleoli appear in each nucleus. The cells are spread out and flattened, and the fat granules are disposed as in the ordinary cell.

The entire observation covered eight hours. The more active part of mitotic division occupied about two hours, but if the initial nuclear changes be included the duration of mitosis is much longer.

The sequence of changes followed in the above cell are in almost all respects similar to those of mitosis many times observed in the mononucleate cell. The only difference is that in the cell described there were to begin with two separate nuclear sacs instead of one. From the presence, in fixed preparations, of bipartite nuclei each portion of which is in a condition of spireme, and the absence, from such preparations, of bipartite nuclei in

which one portion only contains a skein, it is gathered that the prophase in these cells is characterized by the spireme appearing coincidently in the separate nuclear sacs (Fig. 25); later there is formed from the double spireme a single equatorial plate of chromosomes. Though these could not be counted there is no reason for believing that their number was more than that normal for the mononucleate cell.

Not only has mitosis been demonstrated by observation, both on living and fixed preparations, to occur in binucleate cells, but it has been found that it occurs relatively as frequently in these cells as in those with a unipartite nucleus. By making cell counts in the aforementioned 20 preparations from chick heart 375 binucleate cells were found in a total cell count of 41,725. Thus binucleate cells made up a percentage of the total of 0.9, or a ratio of 1 in 111. Among these binucleate cells 2 were found which were in the prophase of mitosis,[1] a percentage of 0.53. Of the mononucleate cells, which numbered 41,106, 47 were in the prophase, or 0.114 per cent. By comparison of these ratios it is found that mitosis occurs 4.65 times as frequently among the binucleate as among the mononucleate cells; allowing for the limited scope of the observation it seems reasonable to conclude that mitosis is as frequent a phase of the life of binucleate cells as of mononucleate, and it would seem that this is their normal method of proliferation. If, in addition, they be considered as dividing by direct fission (for which there is no evidence either from living or fixed material) they would then be possessed of an ability to multiply in excess of that of the mononucleate cells, and there is no reason for supposing this to be the case.

No evidence was brought to light that the separate parts of the bipartite or multipartite nucleus ever combine except during mitosis.

Another interesting observation was made in a fixed preparation, viz., that the early changes in the chromatin which presage the onset of mitosis (*i. e.*, the clumping of the chromatin and its

[1] Prophases alone were counted, in estimating cells in mitosis, since in this stage alone is it possible to distinguish the bipartite from the monopartite nucleus, on account of nuclear fusion in stages later than this in the case of mitosis in the binucleate cell.

segregation into short rodlike masses and finally into a spireme) can take place in a nucleus which is undergoing constriction. Thus it appears that mitosis can proceed as usual in nuclei partially divided or wholly divided by the amitotic process.

Discussion.

The direct division of the nucleus is associated with certain changes of the cell as a whole. Elongation of the nucleus seems to be a prerequisite, and this apparently is secondary to a lengthening and narrowing of the cell body occasioned by the pull of its processes. It has been pointed out that the centrosphere is situated characteristically in a concavity at one side of the nucleus, and, when the nucleus lengthens, this body sinks deeper into its side; at the same time, judging from fixed preparations, and also from the appearance of the living and dividing nucleus, mitochondria come to lie across this narrow nuclear isthmus. These bodies, the centrosphere and associated mitochondria, seem to play a part in the fission of the nucleus. The exact manner of their action is not clear, but it may be that streaming of the nucleoplasm away from the equator of the nucleus follows upon the mechanical irritation of the nuclear membrane by their movements, or possibly upon local alteration of surface tension from their chemical change. Certainly the position which these bodies take with reference to the constricted nucleus points to their participation in the process of fission.

The centrosphere does not divide, nor does it encircle the nucleus as in the form of division described by Meves ('91). The nuclear membrane remains intact and nothing resembling an amphiaster is formed.

Some theories of amitosis have attempted to place the responsibility for the initiation of the divisional stimulus upon the nucleolus, and it has been found by some investigators that in certain nuclei the nucleolus is the first body to become divided. Such a function of the nucleolus does not obtain, however, in the nuclei studied, for, although in some cases there was a division of the nucleolus into two parts, one of which became alloted to each separate nuclear part, yet, sometimes the nucleolus did not divide, and one of the nuclear fragments was without visible

nucleolar material (Fig. 24). Occasionally two fragments would be found in one nuclear part while the other had none; again it was very frequently found—indeed it was the rule—that a nucleus would have two separate nucleolar fragments without any nuclear division. In short not only was the division of the nucleolus in most cases not followed by nuclear fission, but the latter took place in many cases without division of the nucleolus.

Neither before nor after nuclear fission was there discovered an instance of differential staining of the two halves of a nucleus about to be divided, or already divided, as shown by Child ('07) so that there was no evidence of this kind to support the belief of Child that there may be a physiological independence of the nuclear areas even before they become amitotically divided, manifested by a variation in the staining of the two nuclear halves. The study of living nuclei, too, divulged nothing in favor of this hypothesis.

The result of this process of nuclear splitting was the formation of a cell containing one or more separate nuclei of about equal size, and each of about the same size as the nuclei of mononucleate cells. After nuclear fission the separated nuclear elements manifested the power of growth, and seemed to have metabolic independence. The cell protoplasm also of these cells shows an ability to increase in bulk. This is especially evident in giant cells which can thus become quite large.

It is believed, furthermore, that binucleate cells and giant cells in tissue cultures (except foreign body giant cells which arise by fusion of previously separate cells) do not arise in any other manner than that above outlined, for there is no other adequate explanation of their origin. A careful examination of living and fixed material does not reveal any evidence of fusion of cytoplasm without fusion of the nuclei, so that there are no grounds for admitting this as a possible theory of formation. Although many of the binucleate cells undoubtedly do migrate as such from the original piece, where they are doubtless formed in the same manner as they are in the new growth, yet an increase of over 100 per cent. in their number in the growth of the second day as compared with that of the first (as ascertained by making careful counts of the 20 heart specimens aforementioned) can

hardly be explained by the assumption of an increased emigration of these forms during the second day; it is more probable that some of these bipartite nuclei have originated in the new growth, and the observation of this process here confirms this conclusion.

Mitosis, too, cannot account for the formation of these bi- and multipartite nuclei, for in all the cases of mitosis followed through in the living condition the end result has always been two daughter cells, quite separate except for a narrow connecting process, and each containing a single centrosphere. The only theories remaining for consideration are those of nuclear origin *de novo*, or from chromidial extrusions, and these suppositions are too improbable to discuss here. No other process than that of nuclear amitosis, therefore, can account for the production of bi- and multinucleate cells in the cultures examined.

Though the amitotically-divided parts of the nucleus seem to possess metabolic independence, as noted, they do not appear to have reproductive independence, for the reason that they are never dissociated one from the other to become the nuclei of separate cells. Furthermore they show only one type of cell division, viz., mitosis, in which the process begins coincidently in the two nuclear parts, manifested by the simultaneous appearance in each part of a similar spireme. Although in amitosis there is a mass division of the nuclear material there is no meristic division, and it appears that before a cell containing an amitotically divided nucleus can divide it is necessary that the separated chromatin moieties should recombine. This was done in the specimen examined during life, for the combined product of the two nuclear sacs formed a single equatorial plate of chromosomes. Such a type of amitosis, therefore, is not incompatible with the chromosome hypothesis.

Spiremes in bipartite nuclei, and in dumb-bell-shaped nuclei evidently undergoing amitosis, are not confined to the cells of tissue cultures, for Maximow ('08) has described them in the normally-developing mesenchyme cells of the embryo rabbit, and this author refers to similar cell configurations which Karpow ('04) describes in the leucocytes of urodele amphibia.

Fragmentation.

A note may here be made regarding a pathological change which nuclei subjected to unfavorable conditions undergo, viz., fragmentation. This change consists in a breaking up and degeneration of the nuclei. In cells which had grown for six days in unchanged media, in which food and oxygen had become depleted and metabolic products had accumulated (Fig. 26) and also in cells growing in a medium to which a small amount of ethyl alcohol had been added (Fig. 27), this form of degeneration was seen. Multilobulation of the nucleus appeared to antecede the actual breaking away of the parts. These latter were of different shapes and sizes, often did not contain a nucleolus, and showed no power of growth. The cytoplasm enveloping them did not divide and apparently was incapable of increasing.

In preparations containing forms of this kind no mitoses were seen, and the phenomenon seemed to be quite different from nuclear amitosis which occurred only in healthy cells. It is believed, moreover, that the process of fragmentation has been confused with that of amitosis, and it is possible that it is this confusion which has accounted for certain well-known views which regard amitosis as an evidence of degeneration.

Vital Stains.

Finally, a word as to certain so-called "vital stains." It was hoped that gentian violet would prove to be of value in rendering visible the *minutiæ* of the living cell during its vital changes, since the results following the use of this dye recorded by Churchman and Russell ('14) and Russell ('14) with cultures of frog tissue were so encouraging. Unfortunately the dye proved toxic to the tissues used in a dilution of 1 in 200,000, and, though the nucleoli, nuclear membranes, certain granules and the cell borders were brought into sharp relief this staining was always accompanied by cessation of vital phenomena, and the cells speedily went into degeneration.

Janus green, in a dilution of 1 in 80,000, was also toxic, and, while it stained mitochondria specifically, yet these bodies soon became granular and lost their characteristic appearance. Hence neither of these dyes could be spoken of as acting "*intravitam*,"

and they were of value only in obtaining rapidly information as to the obscure structure of the cell; the cell, however, was thus sacrificed.

Summary.

The observations above described and the interpretations thereof may be briefly summarized as follows:

Amitosis was found to involve only the nucleus and was not a method of cell proliferation. It occurred in normal cells and was characterized by a separation of the nucleus into one or more parts which possessed no reproductive independence.

The process of nuclear amitosis consisted in a unilateral or bilateral constriction, manifested by a narrowing of the nucleus in the region of its equator, and a streaming of its contents toward the poles, with final separation of the two nuclear portions. This phenomenon seemed to be associated with the action of the mitochondria and centrosphere upon an elongated nucleus. There was no amphiaster or spireme formation and no centrosome fission. Division of the nucleolus was not an essential. Repetition of this process leads to the formation of a giant cell.

Not all nuclei which show elongation and constriction divide by direct fission, but many return to their usual rounded or oval form. When, however, the constriction has passed a critical point the division goes on to completion, and this final stage is rapid.

Cells containing nuclei in process of, or the result of, amitosis divide by mitosis. Mitosis in binucleate cells, which are the product of nuclear amitosis, is characterized by the simultaneous appearance in the nuclear parts of a spireme, from which a single equatorial plate of chromosomes is formed. Furthermore, binucleate cells divide as frequently by mitosis as do mononucleate cells, and this was the only form of division found to occur in them.

Since the parts of an amitotically divided nucleus do not become separated as reproductive units but divide only by mitosis, in which the chromatin in the parts is recombined, there is nothing in nuclear amitosis opposed to the chromosome hypothesis.

The type of amitosis in which the nucleus is split by the growth

through it from one side to the other of a membrane was not found. Nuclear figures simulating this proved to be caused by the close apposition of separate nuclear sacs, or by nucleoli, mitochondria or folds of the nuclear membrane.

The dyes, janus green and gentian violet, were toxic and their presence in the cell was incompatible with its continued life. They were, however, of service in quickly studying structural details which were not discernible in the living state.

Nuclear fragmentation, which differs in many ways from nuclear amitosis, is a pathological condition, and occurs in degenerating cultures.

It is believed that the facts brought to light through the tissue culture method may be applied to the interpretation of the phenomena of normally developing cells.

LITERATURE CITED.

Child, C. M.

'07 Studies on the Relation between Amitosis and Mitosis. IV. Nuclear Division in the Somatic Structures of the Proglottids of Moniezia. V. General Discussion and Conclusions Concerning Amitosis and Mitosis in Moniezia. BIOL. BULL., Woods Holl, XIII., 165–184.

'11 The Method of Cell-Division in Moniezia. BIOL. BULL., Woods Hole, XXI., 280–296.

Churchman, J. W., and D. G. Russell.

'14 The Effect of Gentian Violet on Protozoa and on Growing Adult Tissue. Proc. Soc. Exper. Biol. and Med., N. Y., XI., 120–124.

Isaacs, R.

'15 A Mechanical Device to Simplify Drawing with the Microscope. Anatomical Record, Phila., Vol. 9, 711–713.

Karpow, W.

'04 (Untersuchungen über direkte Zellteilung.) Inaug.-Diss. Moskau, 1904. *Refer. in* Jahresber. ü. d. Fortschr. d. Anat. (Schwalbe), Jena, n.F., X., 42.

Lambert, R. A.

'12a Variations in the Character of Growth in Tissue Cultures. Anat. Rec. Phila., VI., 91–108.

'12b The Production of Foreign Body Giant Cells in Vitro. Jour. Exper. Med., Lancaster, Pa., XV., 510–515.

Lewis, M. R., and W. H. Lewis.

'15 Mitochondria (and Other Cytoplasmic Structures) in Tissue Cultures. Amer. Jour. Anat., Phila., XVII., 339–401.

Maximow, A.

'08 Ueber Amitose in den embryonalen Geweben bei Säugetieren. Anat. Anz., Jena, XXXIII., 89–98.

Meves, Fr.

'91 Ueber amitotische Kernteilung in den Spermatogonien des Salamanders und Verhalten der Attraktionssphäre bei derselben. Anat. Anz., Jena, VI., 626–639.

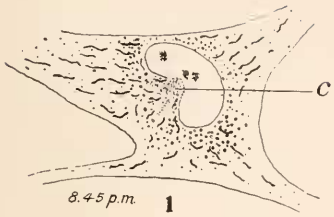
Russell, D. G.

'14 The Effect of Gentian Violet on Protozoa and on Tissues Growing *in vitro*, with Especial Reference to the Nucleus. Jour. Exper. Med., Lancaster, Pa., XX., 545–553.

EXPLANATION OF PLATES.

Plate I.

Figs. 1 to 12. Successive stages covering a period of 2¾ hours in the life history of a connective tissue cell from a 57-hour culture of 5-day chick heart, growing in Locke solution (0.5 per cent. dextrose with extract of chick embryo). The nucleus finally divides by direct fission. Small circles represent fat globules and short threads mitochondria. *c*, Fig. 1, points to the centrosphere. Free-hand drawings × about 900. (Description in text.)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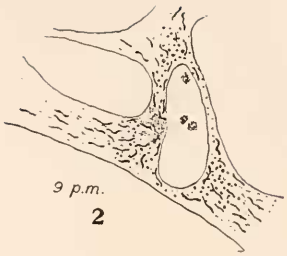
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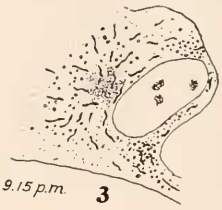
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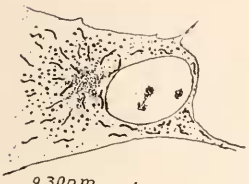
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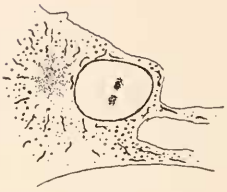
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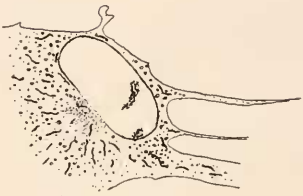
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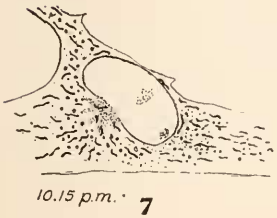
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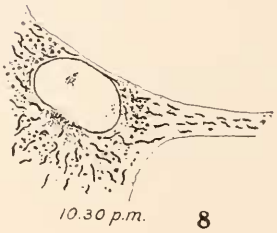
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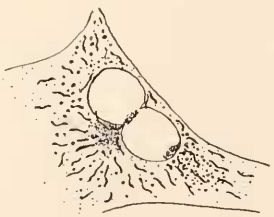
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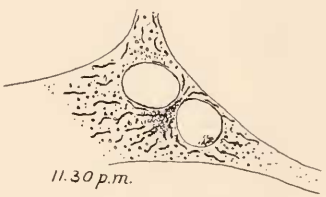
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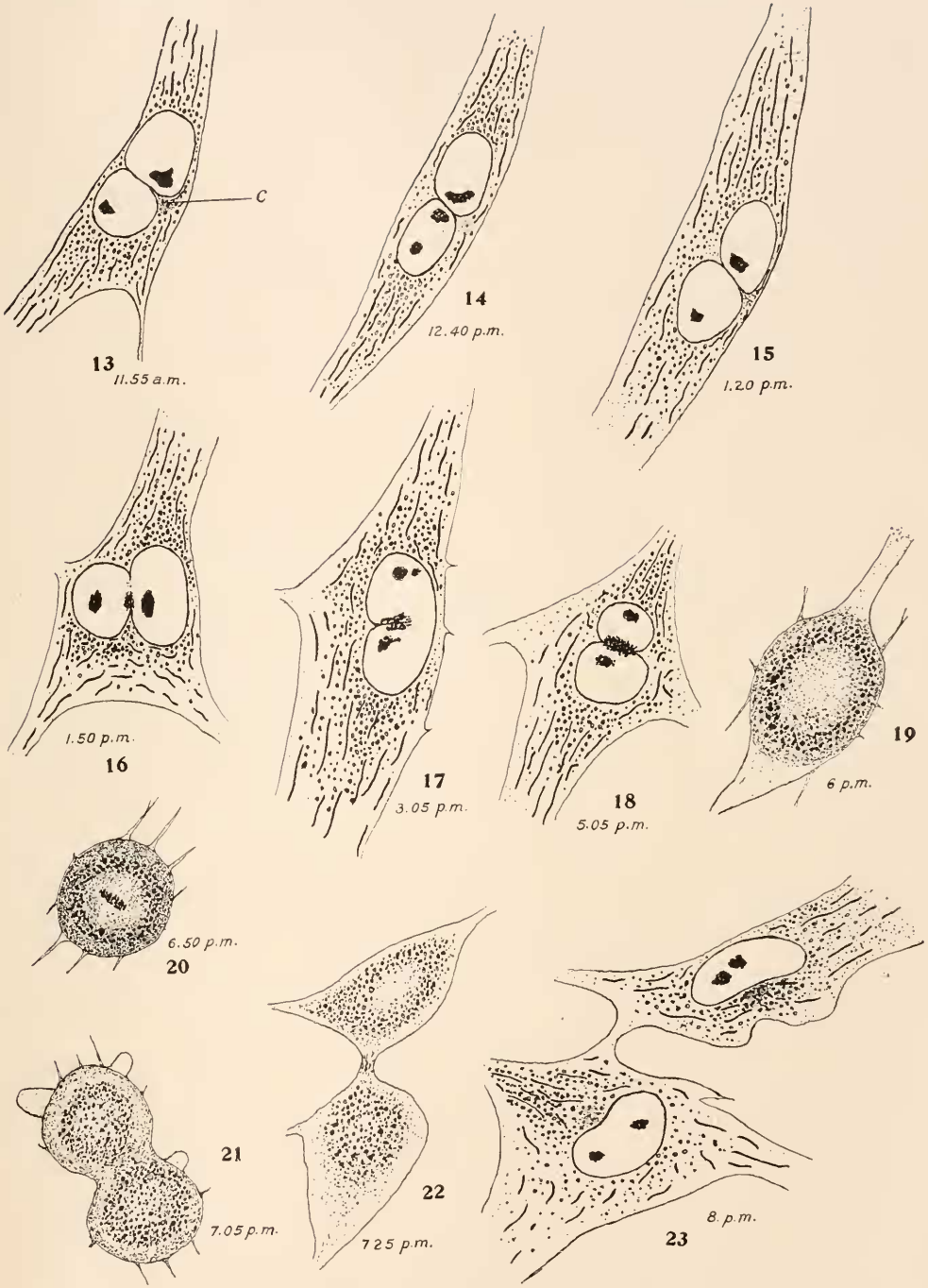
12

C. C. MACKLIN.

PLATE II.

FIGS. 13 TO 23. Graphic record of the process of mitosis in a living binucleate cell, from a 19-hour culture of 7-day chick heart, growing in Locke solution (1 per cent. dextrose with extract of chick embryo). *c*, Fig. 13, points to the centrosphere. Camera lucida drawings. × 1,333. (Description in text.)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8. p.m.
23

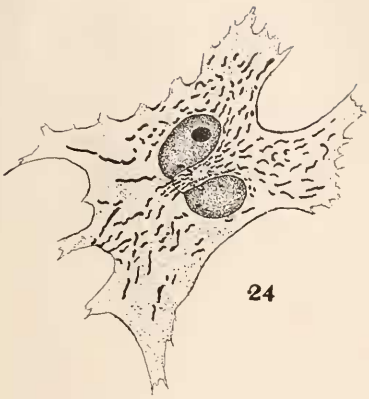
Plate III.

Fig. 24. Direct nuclear division in a connective-tissue cell, final stage. Centrosphere between the nuclear parts. Across the slender filament joining these is a strand of mitochondria. Nucleolus has not divided. Camera lucida drawing from Preparation No. 2, from 5-day culture of 7-day chick heart in Locke solution (1 per cent. dextrose); osmic acid vapor and iron hematoxylin. × 915.

Fig. 25. Spireme in a bipartite nucleus. Prophase of mitosis. Nuclear membrane and nucleoli are disappearing. Camera lucida drawing from Prep. No. 14, 9–1–15 (Lewis collection). Heart from 6-day chick grown in Locke (0.5 per cent. dextrose) with a little yolk; fixed on third day of growth in Zenker; stained with iron hematoxylin (this culture was originally stained with Mallory's connective tissue stain). On account of the method of fixation the cytoplasmic details are not shown. × 915.

Fig. 26. Nuclei showing fragmentation. Camera lucida drawings from Prep. No. 23, 12–1–15 (Lewis collection). 5-day chick stomach in Locke (0.5 per cent. dextrose). Zenker; Mallory connective tissue stain. Culture grown for 6 days in the same media. × 1,012.

Fig. 27. Nuclei showing fragmentation. Camera lucida drawings from Prep. No. 23, 24–11–14 (Lewis collection). 6-day chick stomach in Locke (1 per cent. dextrose) to which ethyl alcohol had been added to make approximately 1 per cent. 3-day culture. Osmic acid vapor and iron hematoxylin. × 1,500.



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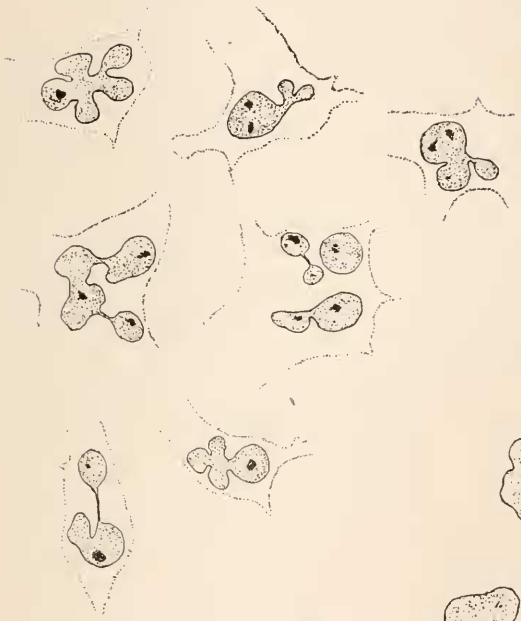

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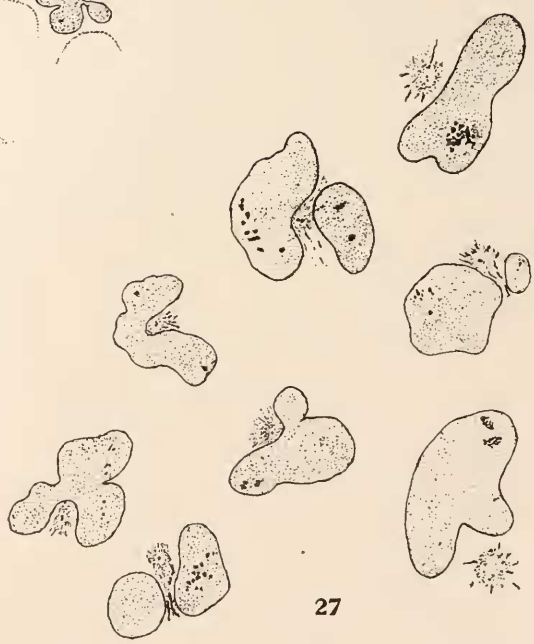

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